ANNUAL REPORT

DIVISION OF INTRAMURAL RESEARCH PROGRAMS

NATIONAL INSTITUTE OF MENTAL HEALTH

October 1, 1991 - September 30, 1992

VOLUME I

SUMMARY STATEMENTS

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Director, Division of Intramural Research Programs

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Nineteen ninety-two has been an eventful year - for the Intramural Research Program (IRP) of the National Institute of Mental Health (NIMH) as well as the other Institutes of the Alcohol, Drug Abuse and Mental Health Administration (ADAMHA). There were changes in leadership of all three organizations; institute goals were redefined and priorities reordered; and there were changes in laws and regulations which affected the very way-of-life of the entire scientific community here on the NIH campus.

Through all these changes, the IRP held steady and continued to fulfill its mission of producing new knowledge to advance the field of neuroscience and contribute to better understanding of the causes, treatment and prevention of the major mental illnesses.

As the 1992 fiscal year comes to a close, the IRP will have come full circle in its organizational history. Along with its parent institute, it will again be a part of the NIH under recent legislation to reorganize ADAMHA. Under the terms of that legislation, the Secretary of the Department of Health and Human Services (DHHS) may not merge the National Institute on Alcohol and Alcohol Abuse (NIAAA), the National Institute on Drug Abuse (NIDA) or the NIMH with any other institute or entity within the NIH for a 5-year period beginning on the date of enactment of the Act. Further, with respect to FY1994 and FY1995, the Directors of NIAAA, NIDA, AND NIMH shall prepare and submit directly to the President for review and transmittal to Congress, an annual budget estimate for their respective institutes after reasonable opportunity for comments by the Secretary of DHHS, Director of NIH and the Institutes' Advisory Council. The President's budget for FY1993 is already firm.

The reorganization of ADAMHA, in addition to transferring the research functions of its three institutes to the NIH (including health services research for each) established a new entity, Substance Abuse and Mental Health Services Administration (SAMHSA) with three Centers, each devoted to providing "services" for its own constituency.

Dr. Bernadine Healy, the recently appointed Director of the NIH, strongly supported the return of the NIMH to "the fold" even though it had become three institutes (NIAAA and NIDA had separated from NIMH) after it left. She and the NIH institutes had been coping with many of the same problems that faced ADAMHA

except on a grander scale. Positions (FTE) were over-committed, travel funds had been cut to the bone, scientists were denied the privilege of accepting honoraria for outside work that had been approved in the past, scientific integrity was no longer an accepted premise and a strategic plan developed for the conduct of research in the future was coming under fire from parts of the scientific community. The long-time Deputy Director for Science had resigned early in Dr. Healy's regime and an extended, intensive search for a successor has just culminated with the appointment of Dr. Lance Liotta formerly Chief, Laboratory of Pathology, National Cancer Institute to this important post. I was privileged to serve on the Search Committee which supplied Dr. Healy with several excellent candidates from whom to choose. Dr. Liotta is a gifted scientist and administrator and his appointment bodes well for the leadership of intramural NIH.

Other contributions I have made this period to the NIH scientific community include service as:

(1) Member of the Search Committee to fill the post of Director of the Clinical Center;
(2) Member of a sub-committee to plan a plenary symposium in connection with the NIH Research Festival for 1992;
(3) Head of the planning effort to honor Nobel Laureate Julius Axelrod with a festschrift;
(4) Member, ADAMHA Senior Biomedical Research Service (SBRS) Credentials Committee, a system recently authorized by the Congress to improve opportunities within the Federal structure to recruit and retain top level scientists by making salaries and benefits more competitive with those of the private sector;
(5) Chair, NIH Women's Committee.

It is worth noting that this last assignment grew out of the fact that the NIMH IRP had played a leadership role in the movement to recognize and take action on a number of matters of concern to women scientists (tenure, promotion, family leave, etc.).

Throughout the year we have persevered in our efforts to strengthen and bring more credibility to the peer review process by which we are evaluated. With the assistance of our able and dedicated Board of Scientific Counselors we believe we have made considerable progress. This is an appropriate juncture to express our appreciation to our "retiring" chairperson, Dr. Bruce McEwen, and to our current board members, Dr. Steve Bunney, Chair, and the following Board members: Dr. Jack Dixon, Dr. Herbert Meltzer, Dr. Edward Stricker, Dr. Susan Leeman* and Dr. Robert Moore*.

* Confirmation of appointment pending

They were joined in their task by ad hoc reviewers selected for their expertise in the work of the laboratory/branch under review. We are deeply appreciative of their efforts.

In the FY1992, the following IRP components were reviewed:

Clinical Neurogenetics Branch
(Detera-Wadleigh only)

Clinical Neuroendocrinology Branch
(Gold)

Section on Pharmacology, Laboratory of Clinical Science
(Saavedra)

Section on Clinical Pharmacology, Experimental Therapeutics Branch
(Potter)

Section on Analytical Biochemistry, Laboratory of Clinical Science
(Markey)

Laboratory of Socio-Environmental Studies
(Schooler)

Laboratory of Neurophysiology
(Wise)

In addition, two Board of Scientific Counselors (BSC) administrative meetings were held aimed at improving and refining the peer review process itself.

The many scientific accomplishments of the past year are detailed in the individual reports of the twenty-three IRP laboratories and branches which follow, and although counting numbers of publications and how many times they are cited are crude and sometimes misleading indicators of scientific productivity, they are nonetheless two objective measures of success. Data covering neuroscience publications and citations (1986-1990) which recently appeared in Science (256:468, 1992), shows the NIMH IRP fared quite well compared to its national (including other NIH Intramural Research Programs) and international colleagues. In fact, when both citations and numbers of publications are considered we ranked ahead of all other comparable research institutions!

Since many members of the IRP staff have received special recognition for their work from their peers outside Government, it is appropriate to note the following:

Dr. Louis Sokoloff received an Honorary Doctorate of Science Degree from Georgetown University.

Dr. David Baron received the Second Annual Nancy C. A. Roeske, M.D. Certificate from the American Psychiatric Association.

Dr. Judith L. Rapoport received the American Psychiatric Association Award for Research from the American Psychiatric Association.

Dr. Ted Usdin received the Young Investigator Award from the National Alliance for Research on Schizophrenia and Depression.

Dr. Robert Post and Dr. Susan Weiss both received the Ziskind-Somerfeld Research Award from the Society for Biological Psychiatry.

Dr. Henrietta Leonard received a Third Prize Young Investigator Award from the Obsessive Compulsive Foundation, Inc.

A number of staff have also received honor awards from the Institute, ADAMHA, the Public Health Service and the DHHS:

Ms. Jean Barr received the NIMH Director's Award for Significant Achievement.

Dr. Carolyn Zahn-Waxler, Dr. Gene Bingham and Dr. Leslie Ungerleider received the ADAMHA Administrator's Award for Meritorious Achievement.

Dr. Karen Berman and Dr. Frank Putnam received the PHS Commendation Medal.

Ms. Brigid Noonan received the ADAMHA Co-worker Award.

Dr. Richard Coppola received the PHS Outstanding Service Medal.

Dr. Rex Cowdry, Dr. Miles Herkenham and Dr. Sanford Markey received the PHS Superior Service Award.

Dr. David Neville, Dr. Thomas Wehr and Dr. Daniel Weinberger received the PHS Distinguished Service Medal.

Dr. Elliot Gershon, Dr. Joel Kleinman and Dr. David Rubinow received the ADAMHA Outstanding Service Medal.

Dr. Philip Gold and Dr. Thomas Uhde received the PHS Meritorious Service Medal.

Ms. Anabel Holliday received the ADAMHA Administrator's Award for Meritorious Achievement.

Dr. Michael Brownstein received the DHHS Distinguished Service Award in Biomedical Research.

Dr. Judith Rapoport and Dr. Dennis Murphy both were named Meritorious Executives in the Senior Scientific Service.

A special note of congratulations to our Clinical Director David Rubinow who was awarded the Clinical Center Award for Teaching (by vote of all the NIH Institutes).

We congratulate them all and share in their pride of a job well-done. (For other recent awards see the Addendum).

It is worthy of note that the Goodwin-Jamison book on "Manic-Depressive Illness" -to which IRP staff made substantial contributions- received the Most Outstanding Book in the Biomedical Sciences Award from the Association of the American Publishers.

It is also worth noting that the IRP prepared and submitted a nominating package for Seymour Kety and Lou Sokoloff to share the 1992 Nobel Prize for their pioneering leadership in the technological advances being made in brain research. The winner will not be announced until November 1992.

Dr. Ronald Schoenfeld completed his first full year on the job after taking over from Hazel Rea, who held the post of Deputy Director for many years. Ms. Rea, who retired, continues to serve the IRP half-time as a re-employed annuitant in the role of Senior Advisor to the Director IRP. Dr. Schoenfeld brings a special interest in neuroethology to the task of recruiting new talent for our Poolesville operation, lately placed under the aegis of Steve Wise, Chief, Laboratory of Neurophysiology. He has also contributed to our recruitment efforts to find a head for a newly created Section on Developmental Neurobiology in the Laboratory of Cell Biology, and for a new Chief of the Laboratory of Psychology and Psychopathology, when Al Mirsky resumes his research career as an independent investigator. Dr. Marian Yarrow is also winding down a long and distinguished career as Chief of the Laboratory of Developmental Psychology. She will continue to serve until a worthy successor can be found.

The IRP is well-served by an able administrative staff under the aegis of Ms. Anabel (Bunny) Holliday who also manages, with the help of a highly competent staff, our budget and personnel operation. The budget has presented special problems this year as, with the help of Dr. David Rubinow, Clinical Director, we have tried to bring under control the accounting complexities and ever-mounting costs associated with running the Clinical Center.

The small hospital that we operate in the William A. White Building at St. Elizabeth's Hospital in the District of Columbia provides a contrast, for -with the assistance of Dr. Rex Cowdry and his administrative staff- we are able to contain hospital costs and still carry out a viable clinical research program for our laboratories and branches located in the William A. White Building.

Activities carried out in connection with the Technology Transfer Act are under the direction of Kathleen Conn, who is assisted in

the task by Newlin Morgan and members of the Technology Transfer Committee chaired by Dr. David Neville. Six Cooperative Research and Development Agreements (CRADAs) were in effect in FY 1992 and another six have been processed and are in final stages of approval. In a related area, patents and Employee Invention Reports (EIR's) maintain a steady flow according to the following summary:

	1992	1991
Patents Issued	2	6
Patents Filed	12	11
EIR's	10	17

Three special committees whose hard-working members contribute invaluable service to the governance of the IRP are: The Animal Care and Use Committee (ACUC) chaired successively this period by Jacqueline Crawley and Brent Stanfield; the Science Promotion Review Committee (SPRC) chaired by Steve Wise and now Rex Cowdry; and the Advisory Committee on Promotions of Research Support Staff (PRSS) chaired by Ted Zahn and now Miles Herkenham. The work of these committees is vital to our successful operation. We note especially that during this period the ACUC helped us deal effectively with a charge brought by People for the Ethical Treatment of Animals (PETA) of improprieties in our care of research animals at Poolesville; the SPRC handled two tenure cases and several promotion cases, all of which passed muster at the NIH Scientific Director's review (thus keeping intact our perfect record). The PRSS also gave its thorough review to a number of support staff promotions. We are indebted to our staff who serve on these committees. They help us maintain the high standards so important to our success. We also welcome to the ranks of our distinguished tenured staff Dr. Esther Sternberg, Laboratory of Neuroendocrinology, and Dr. Susan Swedo, Child Psychiatry Branch.

In a continuing effort to maintain and improve communication within IRP, we reinstated the system of periodic Director Conferences designed to showcase the various laboratory and branch programs with special emphasis on promising young scientific talent. Response from both presenters and audiences has been positive. Also, in an effort to improve intra-divisional communication, we are providing annotated agenda (brief notes) of Laboratory/Branch Chief meetings to all tenured staff. This too has been well-received.

As FY1992 draws to a close, we look forward to occupying the new laboratories reserved for us in Building 49 on which construction is almost complete. The move will affect the entire Laboratory of Neuropsychology and the Veterinary Medicine and Resources

Branch, both of which now occupy sub-standard facilities in Building 9 which cannot be brought up to accreditable standards of AAALAC. Also affected will be the Clinical Neuroscience Branch which moves from Building 10. In addition, present plans call for basic components of the clinical programs housed in the WAW Building at St. Elizabeth's Hospital to move to the NIH Campus in space that opens up as a result of the move into Building 49. We anticipate that new ideas will continue to proliferate as we apply biotechnology to brain functions and diseases and that we will continue to press forward in our efforts to understand memory, sleep, moods and mental illness in all its devastating forms.

ADDENDUM

There have been a number of outside awards which deserve note but which do not fit precisely the prescribed time frame of this report, i.e., October 1, 1991, through September 30, 1992. They are:

Dr. Miles Herkenham received the 1991 Mathilde Solowey Lecture Award from the Foundation for Advanced Education in the Sciences.

Dr. Mortimer Mishkin delivered the 1991 G. Burroughs Mider Lecture at the National Institutes of Health and was named Distinguished Lecturer by the Eastern Psychological Association.

Dr. Robert Desimone received the 1991 Arthur S. Flemming Award from the Downtown Jaycees in Washington, D.C. and the Troland Research Award from the National Academy of Sciences.

Dr. Julius Axelrod received the Decade of the Brain Award from the David Mahoney Institute and the Director's Award from NIH.

Dr. Esther Sternberg received the Commissioner's Special Citation from the FDA.

Dr. Thomas Wehr received the 1991 Anna-Monika Second Prize from the Anna-Monika Foundation.

Dr. Robert Post was a Decade of the Brain Honoree from the National Foundation for Brain Research, and also received a Boots Distinguished Neuroscientist Lecture.

Dr. Richard Wyatt received the Mental Health Bell Media Award from the Mental Health Association of Atlanta, and the Robert L. Robinson Award from the American Psychiatric Association.

Dr. Daniel Weinberger received the 1991 American Psychiatric Association Award for Research from the American Psychiatric Association.

Dr. Seymour Kaufman received the 1991 Hillebrand Prize from the Chemical Society of Washington.

Dr. Thomas Insel received the 1991 Curt P. Richter Prize from the International Society of Psychoneuroendocrinology.

Dr. Seymour Kety received an Honorary Degree from the University of Michigan.

Dr. Andrew Mitz received First Place in the Mid-Atlantic region of The Johns Hopkins 1991 National Search for Computing Applications to Assist Persons with Disabilities from the Johns Hopkins University.

Dr. Howard Nash was elected to membership in the National Academy of Sciences in 1990.

Annual Report of the Biological Psychiatry Branch

National Institute of Mental Health

October 1, 1991 - September 30, 1992

Robert M. Post, M.D., Chief

The Biological Psychiatry Branch consists of several highly interactive clinical and basic science research groups. The clinical studies are focused on the 3-West inpatient unit and each of the three clinical groups has an outpatient component as well. Patients with manic-depressive illness and panic anxiety disorders are studied on the inpatient unit (headed by Dr. T. Ketter). Outpatients with affective disorder (Drs. K. Denicoff and P.J. Pazzaglia) and anxiety disorders (Dr. T.W. Uhde), and those with menstrually-related mood disorder (Dr. D. Rubinow), are also treated and studied in the Ambulatory Care Facility (ACRF).

Each clinical group has a basic science component. Dr. Uhde conducts laboratory studies of neurotransmitter and receptor mechanisms in a genetic strain of anxious pointer dogs. Dr. Rubinow's laboratory efforts derive both from his projects in the Consultation-Liaison Service and studies in patients with affective and menstrually-related mood disorders of neuroendocrine and peptide systems. Dr. S.R.B. Weiss heads a Unit on Behavioral Biology within the Section on Psychobiology, which focuses attention on long-term changes induced by stimulant-induced behavioral sensitization and pharmacological and electrophysiological kindling. Mechanisms of action of the psychotropic anticonvulsant carbamazepine are also studied.

Each of the basic science units is integrally related to the clinical research themes of the Branch. Dr. A. Pert heads the Unit on Behavioral Pharmacology which studies classical neurotransmitter and peptide interactions in relationship to neuropsychiatric syndromes and drugs of abuse. Drs. M. Clark and J. Rosen have acted as co-directors of the Unit on Neurochemistry, studying adenosinergic and "peripheral-type" benzodiazepine receptor mechanisms and the induction of immediate early genes and later reacting neuropeptide genes during environmental, pharmacological, and seizure challenges. Dr. D-M Chuang's Section on Molecular Neurobiology is focusing on the molecular basis of receptor-mediated signal transduction mechanisms (particularly phosphoinositide hydrolysis) and the mechanisms of action of psychotropic drugs, such as carbamazepine and lithium.

Section on Anxiety & Affective Disorders (SAAD), Thomas W. Uhde, M.D., Chief

The SAAD investigates the phenomenology and neurobiology of stress, arousal, fear and anxiety during wake and sleep states. The study of psychosocial and pharmacological treatments for panic disorder and social phobia is also a focus of research. Social phobia is a disabling illness that affects millions of American citizens and, therefore, is deserving of separate scientific attention.

Within the scope of the Section's limited manpower resources, the SAAD investigates the phenomenology of pathological anxiety states in children and adolescents.

The Section also conducts basic neuroscience and behavioral pharmacological experiments in nervous pointer dogs, an animal model that has features similar to both panic disorder and social phobia.

I. Panic Disorder.

Neuroendocrine Studies: The SAAD continues to investigate hypothalamic-growth hormone axis function in patients with panic disorder. As outlined in previous annual reports, the group originally became interested in the growth hormone response to clonidine as an indirect index of post-synaptic α-2 adrenergic receptor sensitivity at the level of the hypothalamus. Four of five subsequent studies have confirmed the finding of a blunted GH response to clonidine in panic disorder patients compared with normal control subjects. While these observations are consistent with a theory of pre-existing noradrenergic hyperactivity (i.e. leading to secondary down-regulation of post-synaptic α-2 receptors), Dr. Uhde has begun to explore other mechanisms that may explain either intrinsic or secondary disturbances in hypothalamic-GH function associated with panic disorder.

Recent findings from the Section indicate that panic disorder patients have blunted GH responses to not only clonidine, but also yohimbine, caffeine, and GRF. In addition, the SAAD has found that patients with panic disorder have a similar tendency toward a delayed rise in GH after glucose compared with normal control subjects. Moreover, unlike depressed patients, patients with panic disorder do not have a paradoxical rise in GH after TRH challenges. Overall, these findings suggest that alteration in hypothalamic-GH function may be a consistent abnormality in panic disorder patients.

Several lines of preliminary evidence from the SAAD also indicate that children with anxiety disorders may have altered growth patterns.

Overall, these findings of the SAAD are consistent with a noradrenergic overactivity model of panic disorder. However, several other mechanisms (See Uhde et al., 1992), may explain the global disturbances in growth hormone secretion that seem to characterize panic disorder. Future research will utilize challenge strategies with CCK-4, pentagastrin, CI-988 (a CCK antagonist), idazoxan, and pyridostigmine.

Chemical Models: The Section has developed several chemical models for the study of panic disorder. In past annual reports, the SAAD reviewed in greater detail the panicogenic effects of caffeine and yohimbine. Both of these panicogenic agents were found to reliably induce a higher rate of panic attacks in panic disorder patients (40-55%) compared to normal control subjects (0-5%). These results suggest a role for noradrenergic and adenosinergic receptor-neuromodulatory systems in the neurobiology of panic disorder.

In a recent study, Dr. Uhde compared the anxiogenic effects of caffeine (480 mg p.o.) after pretreatment with dexamethasone in panic disorder patients compared to patients with major depression and normal control subjects. The depressed patients had a decreased sensitivity to caffeine compared to normal control subjects. Of interest, the panic disorder patients tended to have less severe anxiogenic responses to caffeine with dexamethasone pretreatment compared with prior studies without steroid manipulations. These unexpected findings suggest that the

basal hypercortisolemia associated with major depression (or exogenous dexamethasone) may raise the threshold for caffeine-induced panic attacks.

Dr. Uhde has initiated studies with pentagastrin, a new chemical model, to study the neurobiology of daytime wake and sleep panic attacks. Preliminary findings indicate that pentagastrin produces profound and rapid (within two minutes) onset panic attacks in panic disorder patients. Future research will explore the anxiogenic effects of CCK-4 (a CCK-B agonist) and CI-988 (a CCK-B antagonist) on mood and behavior in panic disorder patients.

Treatment: During the past several years, the Section has investigated a number of different pharmacologic agents (e.g. alprazolam, carbamazepine, clonidine, nifedipine, verapamil). These findings have been published in several peer-reviewed journals.

The Section's most recent investigations have explored the possible anti-anxiety effects of the adenosine reuptake inhibitor dipyridamole. Based on the Section's observations of an increased rate of caffeine-induced panicogenesis (hypothetically via the blockade of adenosine receptors) in panic disorder patients versus normal control subjects, Dr. Uhde explored the possibility that dipyridamole might have antianxiety effects. The findings of this study, however, indicate that dipyridamole has no antipanic efficacy. There are several possible explanations for dipyridamole's apparent lack of antianxiety efficacy: 1) higher doses of dipyridamole might be required to achieve therapeutic levels of adenosine at synaptic sites of action; 2) dipyridamole may not readily cross the blood brain barrier; and 3) dipyridamole-induced increases in adenosine may be too rapidly metabolized. While negative findings failed to confirm the hypothesis that dipyridamole would have antianxiety effects, the results do not rule-out a possible role for adenosinergic mechanisms in the neurobiology of panic disorder. The Section will continue to explore new agents with adenosinergic agonist effects as they become available for use in Phase I studies.

Sleep: Dr. Uhde's group has taken a leading role in the investigation of the neurochemistry and physiology of sleep panic attacks. The Section continues to find that sleep panic attacks occur in a majority of panic disorder patients (60-70%) and emerge from late stage 2 or early stage 3 sleep, possibly during states of increased relaxation. Patients with sleep panic attacks develop two different patterns of fear response to their sleep panic attacks. 1) Some patients tend to develop mild or nonexistent avoidance of sleeping or 2) to be impaired to a significant and severe degree. The latter group becomes chronically sleep deprived, probably resulting in an exacerbation of their anxious symptomatology. The group has previously demonstrated that 24-hours of total sleep deprivation worsens the course of illness in approximately 60% of panic disorder patients.

The Section has or will be initiating a series of studies investigating the behavioral and biochemical effects of panicogenic agents such as caffeine, yohimbine, pentagastrin, CCK-4, and idazoxan *during sleep* in panic disorder patients versus normal control subjects.

II. Social Phobia.

The Section continues to investigate the neurobiology, physiology and pharmacotherapy of social phobia. In past annual reports, the Section has reported that social phobic patients have normal hypothalamic-pituitary-adrenal

and hypothalamic-pituitary-thyroid axes function as evidenced by normal basal levels of plasma cortisol, normal mean 24-hour urinary free cortisol, normal cortisol suppression from dexamethasone and normal plasma levels of T3, T4, TBG, TSH and normal titres of antithyroid antibodies.

The group has been actively involved in the study of growth hormone (GH) function in social phobic patients. Similar to our findings in panic disorder patients and patients with major depression, social phobic patients have a significantly blunted GH response to *intravenous* clonidine. However, recent unpublished findings from a study conducted at the University of North Carolina by a former fellow in the Section, Dr. Manuel Tancer, failed to find a blunted GH response to clonidine with oral clonidine in patients with social phobia. Differences in methodology may contribute to these discrepant findings. Overall, however, it appears that the degree of GH blunting to clonidine in social phobia is less consistent and less impressive than the decrements observed in panic disorder patients. The Section continues to explore the clinical and biological predictors of blunted GH responses in neuropsychiatric patients and continues to explore the hypothesis that blunted GH responses to clonidine may identify patients with tricyclic responsive syndromes.

Chemical Models: The Section has demonstrated that social phobic patients have an increased anxiogenic response to caffeine compared with normal control subjects, although, by direct comparison, less severe than that observed in patients with panic disorder.

Sleep: Standard polysomnographic variables have been investigated in patients with social phobia. In contrast to panic disorder patients those with social phobia have normal indices of sleep. These objective findings parallel the subjective experience of patients with social phobia, who report low rates of insomnia or sleep-related anxious arousals from sleep.

Treatment: The Section has previously reported on the value of both cognitive-behavioral therapy and pharmacotherapy (i.e. phenelzine & alprazolam) in the treatment of social phobia. At the present time, the Section is investigating the relative efficacy of imipramine hydrochloride versus phenelzine in the treatment of social phobia. As part of this project, the Section will be investigating the hypothesis that blunted GH responses to clonidine will identify a group of heterogenous anxious patients, including social phobics, who have a positive response to tricyclic antidepressant medications. The Section will also be initiating studies to investigate the efficacy of CI-988, a CCK-B antagonist, in the treatment of social phobia.

III. Animal Research:

Nervous pointer dogs have been suggested as a model for panic disorder and social phobia. At the present time, the major focus of interest with this model is the investigation of hypothalamic-GH function. These studies parallel our research in humans with anxiety disorders. The following findings highlight the work with this animal model.

1) Adult nervous dogs, especially females, have disturbances in growth and alterations in weight and stature compared to normal dogs of the same pointer breed.

2) Nervous pointer dogs have decreased levels of insulin-like growth factor I (IGF-I) compared with normal pointer dogs.

3) As expected, height and weight are predictors of IGF-I levels in the dog colony. However, the *best* predictor of IGF-I levels is the ratings of fearfulness. The greater the ratings of fearful behavior, the lower the IGF-I levels.

4) Nervous pointer dogs have increased rates of mitral valve disease compared with normal pointer dogs.

Overall, these findings parallel many of the observations made in humans with panic disorder. The alterations in size and low levels of IGF-I suggest that the neurochemical substrates of alarm, fear, and arousal may influence hypothalamic-GH-somatomedin function. Future studies will determine whether genetic strains of GH deficient mice/rats have an increased sensitivity known panicogenic agents in panic disorder humans.

Section on Psychobiology (Dr. R.M. Post, Chief)

Lithium-refractory Bipolar Illness

This Section has as its primary focus the investigation and treatment of patients with refractory unipolar and bipolar affective disorders. Patients are studied on the 3-West Clinical Research Unit under the direction of Dr. Terence Ketter, where they undergo an extensive series of baseline, medication-free evaluations and double-blind, placebo-controlled acute and prophylactic clinical trials with novel and standard psychotropic agents. A particular focus is on better understanding and treatment of patients with rapid cycling and refractory bipolar disorders. The bipolar population in general has been gravely understudied in the past decade, in part because of the illusion that adequate treatment existed for the vast majority of bipolar patients. In fact, recent data suggest that there are increasing numbers of patients who are lithium-refractory, and recent estimates from Per Vestergaard and colleagues indicate that as many as 60% of unselected patients where lithium treatment is attempted do poorly. There is increasing recognition that many subtypes of patients with bipolar illness are particularly lithium-refractory. These include patients with dysphoric mania, rapid cycling, a negative family history for affective illness in first-degree relatives, a pattern of illness characterized by depressions switching into mania followed by a well interval (D-M-I), and patients with a history of co-morbid drug and alcohol abuse.

We have recently uncovered two novel routes to lithium-refractoriness as well. These include the development of apparent tolerance to the positive psychotropic effects of lithium in long-term prophylaxis, even in patients who show initially good responses in the first years of treatment. In addition, we have documented the phenomenon of lithium-discontinuation-induced refractoriness, in which patients show long periods of sustained response to lithium, discontinue treatment, experience a relapse, and fail to re-respond to the drug once it is reinstituted. These data are convergent with the recent data of others that having more than three or four prior episodes of affective illness before starting lithium prophylaxis may convey a lesser degree of response. Thus, in our discontinuation-induced refractory patients who were well maintained, the experience of a new epi-

sode may not only be associated with the potential morbidity of that episode, but it may also provide an additional episode which then is associated with refractoriness to what was previously effective treatment.

Mechanisms Underlying Cycle Acceleration and Transition from Precipitated to Spontaneous Episodes

These data can also be viewed in the context of the kindling and sensitization models explicated in the clinical laboratory by Dr. Susan Weiss. These phenomena suggest the possibility that episodes of affective illness may facilitate subsequent episodes, either increasing the likelihood of a recurrence, or increasing its severity. In addition, we have recently completed a metanalysis which indicates that initial episodes of unipolar and bipolar affective illness are often associated with psychosocial precipitants, but with greater numbers of recurrences the direct relationship to psychosocial stressors becomes less evident. Thus, while initial episodes are likely to be precipitated, with multiple recurrences episodes may occur more autonomously or "out of the blue". Thus, the tendency for cycle acceleration in the course of illness is accompanied by an increased degree of automaticity, two phenomena originally described by Kraepelin and now re-documented in systematic controlled studies in the literature and observed in patients with refractory mood disorders on our Unit.

Clinical findings that stress impact on immediate early genes, such as the proto-oncogene c-fos observed in our laboratory by Takashi Nakajima, and the observation that a greater spatio-temporal distribution of c-fos is evoked in the course of amygdala-kindled seizures (as observed by Clark), have led to the formulation that psychosocial stresses involved in the precipitation of affective illness may leave behind residual vulnerabilities to reactivation of affective episodes by impacting on gene expression. Jeff Rosen has observed a close correspondence in space and time of the induction of mRNA for TRH to the induction of c-fos during amygdala-kindled seizure evolution. In addition, Mark Smith and associates have observed transient expression of mRNA for CRH in the amygdala-kindled paradigm as well. These data suggest that amygdala-kindled seizures may be associated with the induction of immediate early genes such as c-fos, which then could have a longer-lasting impact on neuropeptide systems and other neurotransmitter and receptor adaptations. Moreover, in kindling, not only changes in microsynaptic structure have been observed, but also more macroscopic changes in synaptic sprouting and, in some instances, cell loss have been noted by the group headed by Sutula and associates. In the process of kindled seizure evolution, not only do seizures become more readily inducible and animals become responsive to stimulation that was previously subthreshold, but with repeated induction of amygdala-kindled seizures, a phase of spontaneity occurs. Thus, there appears to be a reorganization of the kindled "memory trace" to the point where exogenous electrophysiological stimulation is no longer required to induce seizures. In a parallel fashion, a similar transition may be occurring in the affective disorders, where episodes are initially triggered by psychosocial stresses and, following sufficient numbers of recurrences, begin to occur autonomously. In this fashion, one could conceptualize how acute psychosocial stress might, as in the kindling model, be transduced into a longer-lasting neuropeptide alteration via effects on gene expression.

It is also conceivable, as described in other models of learning and memory, that changes could also occur at the level of synaptic microstructure based on

prior experience as well. These data may help explain the long-lasting vulnerability to disorders with lesser degrees of psychosocial stressor induction and, additionally, suggest, as in the sensitization model, that the occurrence of multiple episodes themselves might be associated with increased likelihood for future episodes or equal or greater magnitude. Such a formulation emphasizes the potential impact of stresses and affective illness at the level of gene expression and helps to support the growing clinical and empirical data base suggesting the importance of early and sustained intervention for prophylaxis in the unipolar and bipolar affective disorders.

Life Charting, Ultra-rapid Cycling, and Chaos Theory

We have been able to uncover the phenomenon of lithium discontinuation-induced refractoriness because of the systematic use of life-chart techniques, adapting them from the early version utilized by Kraepelin and expanding their systematic retrospective and prospective scope. Using a variety of techniques over more refined time domains, we have additionally described novel phenomena of ultra-rapid and ultra-ultra-rapid (ultradian) cycling not previously described, in patients with classical bipolar disorder. These data suggest that cyclicity in bipolar illness follows some of the same rules as fractal geometry, where there is self-similarity in pattern in different dimensions of temporal or spatial resolution. The ultradian cycle frequencies appear chaotic just as the more macroscopic cycling frequencies do. In many patients who have presented at the NIMH with documentable ultradian cycling, we have observed, over the course of their illness, a progression of cycling frequencies from isolated intermittent episodes, to regular continuous episodes, to more chaotic patterns. These progressive phases of cycle acceleration have recently been mathematically modeled by Mark Jones and Mark George, suggesting that some of the patterns of cyclicity may be deterministic and follow mathematical principles derived from chaos theory.

Response to Anticonvulsants in Bipolar Illness

These changes in the temporal patterns of course of illness may relate not only to changes in the underlying neurochemistry, but also in pharmacological responsivity. As noted above, many patients with rapid cycling disorders that often occur late in the course of illness are inadequately responsive to lithium carbonate, and we have documented that these patients are often responsive to the anticonvulsants carbamazepine and valproate. We have documented good acute antimanic responses to carbamazepine studied in a double-blind fashion in 62% of patients, although only 17 of 57 acutely depressed patients (31%) have shown substantial antidepressant responses to double-blind administration of carbamazepine monotherapy. Nonetheless, approximately 50% of refractory bipolar patients in our studies and in the literature have good long-term response to prophylactic treatment with carbamazepine against the recurrence of both manic and depressive episodes. Yet, as observed for lithium carbonate, a subgroup of patients (particularly those with the most rapid deterioration in their course of illness in four years prior to administration of drug) show a pattern of loss of efficacy (tolerance) to the positive effects of carbamazepine in the second, third, or fourth year of treatment. We have also observed occasional loss of efficacy in patients who are initially highly responsive to long-term prophylaxis with valproate. Thus, we have highlighted the new phenomenon of the development of tolerance to the spectrum of effective psychotropic agents in the long-term treatment of the most malignantly cycling patients with bipolar disorder.

Approaches to the Development of Tolerance

Based on preclinical models and empirical trials, we are attempting to devise strategies for treating these patients with extremely refractory mood disorders either based on tolerance or initial nonresponsiveness. Dr. Weiss' model of contingent tolerance to the anticonvulsant effects of carbamazepine (see her more detailed description below) suggests that the subjects may re-respond to a drug to which they have become tolerant following a period of medication discontinuation. We have documented this type of renewed responsiveness in several case studies that support the possibility that tolerance development to the psychotropic effects of carbamazepine and related agents may also be occurring on a contingent basis, and be amenable to some of the same manipulations that are effective in reversing tolerance in the preclinical models. These models also suggest that once animals have become tolerant to carbamazepine, which acts through so-called peripheral-type benzodiazepine receptors, they are cross-tolerant to other drugs acting through these receptors, such as PK-11195, but not to different agents that act at other receptors, such as diazepam, which is active at central-type benzodiazepine receptors. Should these preclinical data be extrapolatable to observations in man, they suggest the potential utility of switching tolerant patients to drugs with novel mechanisms of action. However, Dr. Weiss observed cross-tolerance between carbamazepine and valproate, suggesting that these two anticonvulsants, thought to exert their mechanisms of action in seizure disorders by different mechanisms, may share a common neural substrate in tolerance development to psychotropic effects as well. Preliminary studies in collaboration with Mike Clark suggest that contingent-tolerant animals may show a decreased up-regulation of $GABA_A$ receptors compared with non-tolerant animals, which could account for the cross-tolerance to valproate. Much clinical and preclinical work remains to develop a useful clinical algorithm for the management of patients who have become tolerant to the long-term effects of the major psychotropic drugs used in the treatment of refractory bipolar illness -- i.e., lithium, carbamazepine, and valproate.

Differential Response to Anticonvulsants

In patients who are not tolerant to these agents, we have documented differential response among the anticonvulsants. Some patients respond to carbamazepine and not valproate, as well as vice-versa. In addition, Dr. Ketter has documented the first patient studied with double-blind, placebo-controlled methodology who showed inadequate response to either carbamazepine or valproate alone, but a dramatic response to both when used in combination. These data are consistent with an extensive parallel literature with this combination in the treatment of refractory seizure disorders. Taken together, these data suggest the possible synergistic effects of these two agents, findings which deserve further mechanistic dissection.

Nimodipine in Affective Illness

Most recently, we have been exploring the potential psychotropic effects of the calcium-channel blocker/anticonvulsant nimodipine. Five of the first 12 patients have shown some evidence of clinical response to this agent. Most interestingly, some of the patients with the most ultra-rapid cycling were among those who showed positive responses. In two instances, clinical responses were documented by not only successful treatment with the substitution of nimodipine for placebo, but relapse on placebo substitution and re-response once nimodipine was reinstituted on a blind basis in a B-A-B-A design. One of these responsive

patients showed recurrent brief depression, further supporting the preliminary observations that patients with the most paroxysmal onsets and off-sets of their mood disorder were among those responsive to the calcium-channel blocker nimodipine. Clearly, further work is required to follow up on these promising initial observations suggesting not only possible new treatment modality, but a novel mechanism to target for further therapeutics; i.e., calcium channel fluxes and dysregulation. These observations are all the more intriguing with the suggestion that carbamazepine may, in part, be exerting some of its therapeutic actions through a calcium channel mechanism either directly or through effects on peripheral-type benzodiazepine receptors or adenosine or $GABA_B$. However, it is also well recognized that carbamazepine exerts potent effects on sodium as well as potassium channels, raising further questions about these possible ionic mechanisms for carbamazepine's psychotropic effects as well. It is unlikely that the sodium channel blocking effects of carbamazepine do account for its positive effects in manic-depressive illness as these are shared by phenytoin, and this agent has not been observed in our studies to be effective in any of the first five patients treated with phenytoin in a double-blind fashion.

Mechanism of Action of Carbamazepine

In addition, carbamazepine exerts effects on a variety of neurotransmitter-neuropeptide systems that remain candidates for psychotropic efficacy. In order to dissect whether putative $GABA_B$ agonist effects of carbamazepine, which are thought to account for its positive effects in trigeminal neuralgia, could also account for its psychotropic effects in manic-depressive illness, we conducted a clinical trial of l-baclofen in patients with affective illness. In the first five patients studied, not only did we not observe positive therapeutic effects, but exacerbations were observed in three patients, with improvement following placebo substitution. These data are convergent with the view that $GABA_B$ agonistic effects are not likely to be associated with positive psychotropic effects, and suggest the utility of a $GABA_B$ antagonist in refractory illness, a tack which we will be pursuing in the near future.

Similarly, carbamazepine exerts positive effects on peripheral- rather than central-type benzodiazepine receptors. In order to study whether actions through this peripheral-type benzodiazepine receptor could account for positive psychotropic effects in their own right, we have completed an approved protocol for the agent alpidem, which works potently (although not selectively) at peripheral-type benzodiazepine receptors, and hope to initiate this clinical trial in the near future. Carbamazepine also exerts a multiplicity of effects on neuropeptide systems, each of which is a candidate for carbamazepine's psychotropic effects. Carbamazepine appears to act as a vasopressin-like agonist; it decreases somatostatin in the CSF of patients chronically treated with the drug, it increases substance P levels and substance P receptor sensitivity (in preclinical studies by others), and has complex interactions with opiate peptide systems.

In an attempt to narrow the potential field of neurotransmitter ion channel neuropeptide effects of carbamazepine that may be related to its psychotropic effects, we have utilized a model of local anesthetic-induced seizures in rodents, which requires chronic administration of carbamazepine in order to show efficacy. Thus, this model parallels the clinical data in which chronic carbamazepine appears to be required in order to demonstrate acute anti-manic and antidepressant effects of the drug.

Combination Therapy and Pharmacokinetics of Bupropion

While acute and breakthrough episodes of mania during prophylaxis can most often be readily treated with lithium, carbamazepine, valproate, or adjunctive benzodiazepines or neuroleptics depressive breakthroughs remain more problematic in the long-term treatment of bipolar patients. Thus, we have begun to study a variety of adjunctive and combination treatment approaches to refractory bipolar depression. In efforts headed by Dr. Ketter, the clinical efficacy and pharmacokinetic interactions of carbamazepine and bupropion have been studied. Bupropion appears to exert its antidepressant efficacy through novel mechanisms involving increases in dopamine in n. accumbens and striatum, and may not be associated with the same degree of mania and cycle acceleration as other, more traditional, unimodal antidepressant agents. Carbamazepine appears to markedly decrease levels of the parent bupropion compound and markedly increase levels of its principal metabolite hydroxy-bupropion. At the same time, levels of threo-hydrobupropion decrease and erythro-hydrobupropion are relatively unchanged. These profound pharmacokinetic interactions do not appear to be associated with deleterious effects, and the relationship to the degree of antidepressant efficacy of the parent compound and metabolites remains to be further elucidated. In contrast to these major pharmacokinetic interactions with carbamazepine, bupropion is relatively unaffected when used in combination with valproate.

Effects of Thyroid Manipulation in Affective Illness

Thyroid augmentation strategies, particularly with T_3 (25-50 μg), have also been used successfully by our group in the treatment of refractory bipolar patients. The T_3 data, the preliminary observations of the potential efficacy of intravenous TRH, and a variety of other theoretical rationales, led us to initiate a trial of intrathecal TRH in refractory depressed patients in order to deliver adequate quantities of drug to the CNS and circumvent the blood-brain barrier. The first patient has been administered intrathecal TRH without side effects, and the potential clinical efficacy of this novel treatment intervention, and specific test of the role of TRH in refractory affective disorders, awaits further systematic controlled observations. It is noteworthy in the preclinical models of Dr. Weiss that it was observed that, paradoxically, a period of time without the induction of amygdala-kindled seizures led to a relative loss of efficacy rather than an enhancement of the anticonvulsant effects of carbamazepine. A variety of data suggest that seizures may be inducing an anticonvulsant principle that was effective in itself or enabled the anticonvulsant effects of carbamazepine. TRH remains a potential candidate for this effect, since it appears to be released with amygdala-kindled and electroconvulsive seizures and has been shown to be anticonvulsant (by others) when administered to animals or man. These observations led to the formulation that some of the neurobiological changes observed in the process of kindling may be related to the primary pathological process ("bad guys"), while others are secondary and compensatory, potentially exerting anticonvulsant effects ("good guys").

Based on these preclinical observations, we have theorized with Dr. Weiss that there may also be a similar spectrum of neurobiological changes in the affective disorders, some relating to primarily pathological changes, and others to secondary or compensatory changes. This work was awarded the Ziskind Somerfeld Prize of the Society of Biological Psychiatry. This formulation provides a novel targeting for therapeutics, highlighting the importance of making the distinction

between abnormalities which represent the "bad guys" versus the "good guys. In one instance, therapeutics should be aimed at ameliorating the pathological process but, in others, the biological alterations should be enhanced. These data may be consistent with the observations that sleep loss in depression may be, paradoxically, compensatory or therapeutic, as evidenced by the high degree of clinical responsivity and acute antidepressant responses to one night's sleep deprivation in acutely depressed patients. If sleep loss in depression were primary and pathological, one would expect further deprivation of sleep to be associated with exacerbation rather than improvement in mood, and this is obviously not the case. Moreover, preliminary observations in our group have been consistent with a wider literature suggesting the potential relationship of the degree of improvement in sleep disordered patients to the degree of increase in TSH evoked by one night's sleep deprivation. This observation, which continues to be observed on a trend level, deserves further exploration in both its own right, as a potential marker for degree of clinical response, and as a further piece of data incriminating alterations in the thyroid axis in the pathophysiology of depression and its amelioration by one night's sleep deprivation.

Deborah Shapiro and Dr. Ketter have further documented novel findings using sleep deprivation as not only a therapeutic tool, but as a probe of the neurobiology of an affective episode that may change as a function of duration of depression. These investigators documented that many bipolar patients with recurrent depressions of fairly regular durations are relatively refractory to the therapeutic effects of one night's sleep deprivation early in the course of a depressive episode, but show more dramatic or sustained effects toward the mid-phase of the episode; they may even show a complete antidepressant response and triggering of the end of the episode by sleep deprivation when it is instituted relatively late in the course of a depressive episode. These observations suggest that the neurobiology of a depressive episode may change as a function of its duration. Initial observations in our patient population also suggest that it is the patients who show a more robust diurnal variation in mood, who are the most likely to show robust responses to sleep deprivation.

Limbic Activation with Procaine: Brain Imaging Studies

Evidence in animals suggests that local anesthetics selectively activate structures in the temporal lobes and limbic system. Based on these observations, we have been utilizing intravenous administration of the local anesthetic procaine in man to assess and probe limbic system responsivity in normal volunteers and patients with affective and borderline personality disorders. Similar to the findings with electrical stimulation of the structures in the deep temporal lobe by others, we have observed dose-related psychosensory alterations with procaine administration. These have been associated with a range of mood alterations from euphoric to dysphoric. The degree of dysphoria was correlated with the degree of fast activation in the EEG over temporal lobe areas. We questioned whether the fast EEG activity over the temporal lobe might not be muscle artifact, leading us to consider alternative brain imaging techniques to document procaine-induced activation of temporal lobe structures. This has been accomplished most recently under the direction of Dr. T. Ketter using O^{15} blood flow techniques. Following procaine administration, he has observed increases in blood flow in anterior, temporal, and orbital frontal areas, as well as in the cingulate gyrus in both normal volunteers and patients with affective disorders. Patients with cocaine abuse disorder with and without panic will also be studied. These areas appear to be

activated independently of the positive or negative valence of the mood changes induced, and further analysis is required in order to discern whether specific neural substrates are associated with a given type of affective responses. Nonetheless, these data confirm that procaine activation is selective for temporal lobe and related limbic neural substrates and should prove valuable in testing the hypothesis that there are differential limbic responsivities across different psychiatric illnesses and that these might be related to the degree of psychotropic response to the anticonvulsants. So far, systematic data have been lacking linking the degree of psychotropic response to the anticonvulsants with any measure or temporal lobe or limbic dysfunction based on EEG, psychosensory, or PET data. Given the crucial need to match individual patients with appropriate drugs to which they would be acutely and long-term responsive, systematic exploration of the potential link of procaine activation to degree of clinical response remains a promising area for further investigation.

PET deoxyglucose studies in affectively disordered patients on and off carbamazepine failed to document specific "hot spots", potentially indicative of limbic areas showing increased activation in the absence of procaine. Rather, there is some evidence for relative hypometabolism in temporal lobe structures associated with the affective disorders similar to that observed in interictal PET scans of seizure patients studied by others. During carbamazepine administration, there appear to be further decreases in regional cerebral glucose utilization, rather than increases in most areas of brain.

Under the direction of Mark George, a series of studies have been initiated in order to discern what areas of brain studied with O^{15} PET techniques might be activated by different psychological tasks and whether this degree of activation may be abnormal in patients with affective disorders. The first series of tests involve the differential study of recognition of facial identity versus facial emotional expression, as the latter has been demonstrated to be deficient in patients with primary affective disorders. Facial identity appears to activate temporal lobe structures, in the studies of others, but the neural substrate for recognition of emotional faces has not been previously elucidated in man; it is hypothesized to reside in more anterior portions of the temporal lobe based on studies in primates.

We will follow up psychological probes of putative temporal lobe function with other studies with O^{15} PET scan techniques during performance of the Stroop test (which activates the cingulate gyrus in normal volunteers). Thus, using these various neuropsychological activation paradigms, we hope not only to elucidate neural substrates involved, but also discern possible abnormalities in patients with affective disorders.

Initial data from MRI scans additionally implicate abnormalities in temporal lobe structures in patients with affective disorders where we have observed evidence of decreased area and volume of the temporal lobe compared with normal volunteers. These preliminary findings are being followed up in larger studies. Other approaches to the neurobiology of the affective disorders in our Section focus on the use of CSF analysis in patients studied during ill and well stages as well as on and off psychotropic drugs. As previously noted, Rubinow and associates have found evidence of decreased CSF somatostatin in depression which normalizes on recovery from the illness. We are currently analyzing data to ascertain

appeared to respond to fluoxetine with significant reduction or elimination of their PMS symptoms; the ability of response to m-CPP to predict response to fluoxetine awaits analysis of the m-CPP data.

2. Menopause-Related Mood Disorders

Ten women have completed and 13 entered a double blind placebo controlled trial of estrogen in the treatment of perimenopausal depression in the absence of hot flushes. This patient group was selected in order to avoid confusion of a primary effect of estrogen on mood with an effect secondary to estrogen treated hot flushes. Thus far a significant beneficial effect of estrogen on affective symptoms (sadness, tearfulness, worry) as well as sleep disturbance and fatigue has been identified. The effects of estrogen on cognitive function await analysis of the collected data. Twenty-four hour physiologic monitoring has been performed in these patients in order to identify the effects of estrogen on thermoregulation in patients who do not complain of hot flushes. Similar monitoring has been performed in four patients with hot flushes and four perimenopausal controls. TRH infusions in seven patients with perimenopausal depression and seven controls have revealed no abnormality of response. Oral m-CPP challenge tests are being performed to evaluate the endocrine and thermal response to a serotonergic agonist in perimenopausal women. Performance of the clomiphene citrate challenge test demonstrated a significantly increased FSH and significantly decreased estrogen response in women with irregular menstrual cycles compared with age matched normal cycling women. These changes in women with new onset irregular cycles are particularly interesting given our observation of the reversal of depression and hot flushes and normalization of FSH levels in perimenopausal women who spontaneously resume normal cycling.

3. Hormonal and Peptide Studies in Affective Disorders

Somatostatin - CSF studies (which have documented decreases in somatostatin in depression and normalization with recovery) have continued in a variety of other neuropsychiatric populations. Forty-five patients with Alzheimer's disease and 12 age matched controls have been studied as part of the longitudinal effort to determine the relationship between CSF somatostatin and clinical deterioration in Alzheimer's disease. Fifty-seven violent offenders were compared with 27 controls, with no diagnostic group-related differences observed. A 30 hour CSF study was performed in collaboration with M. Kling and revealed progressive elevation of sample concentrations over time, perhaps suggestive of an effect of the sampling procedure on CSF neuropeptide concentrations. CSF somatostatin has also been measured in patients with Sydenham's Chorea and controls (collaborator S. Swedo) as well as in patients with Eosinophilia Myalgia Syndrome (EMS) and controls (collaborator M. DeBellis).

Beta Endorphin - CSF beta endorphin has been measured in patients with EMS and those with affective disorder during treatment with idazoxan or fluvoxamine (collaborator M. DeBellis). Additionally, Rubinow and associates have been measuring beta endorphin as the secretory outcome measure of IL-2 stimulation in AtT20 cell cultures.

4. Studies in Behavioral Medicine

In a prospective study of anabolic steroid (AS) use in normal volunteers, this group has demonstrated significant decreases in reproductive endocrine axis hormone concentrations as well as evidence of decreased thyroid axis activity. Ratings of AS-induced distractibility were significantly correlated with increased

plasma cortisol, plasma DHEA, and CSF ACTH as well as with blunted AS-induced testosterone suppression. Blunted effects of AS on reproductive hormones (testosterone, DHT, estradiol, SHBG, LH) were associated with increased cognitive impairment and negative (distressing) symptoms as well as with decreased positive symptoms (e.g. energy, confidence) during high dose AS administration. Finally, anabolic steroids produced significant increases in IL-2 with decreases in IL-1, providing some of the first evidence of their dissociated secretion. These data may help identify some of the biological mechanisms underlying both the adverse behavioral and health consequences of anabolic steroids. Ongoing behavioral medicine studies include the following: 1) evaluation of the antidepressant efficacy of methylphenidate in the medically ill; 2) detection of conditioned immunomodulation in a study employing gamma interferon as the unconditioned stimulus; 3) evaluation of the physiologic and endocrine characteristics of central hypothyroidism before and after thyroid replacement; 4) description of the presenting characteristics of women with pudendal neuralgia and their response to tricyclic antidepressants.

Unit of Behavioral Biology (S.R.B. Weiss, Ph.D., Chief)

The major emphasis of this unit has been the elucidation of the phenomenology and biological substrates of sensitization, tolerance, and kindling. These particular preclinical models have the advantages of permitting analysis of short and long term changes in behavior, biochemistry and physiology, and also determination of associative or learning mechanisms that can dramatically affect the behavioral and physiological outcome of the experimental manipulations. The kindling, sensitization, and tolerance paradigms are used to study the long-term consequences and evolution of progressive behavioral and physiological alterations in rodents following repeated administration of pharmacological or electrophysiological stimuli. These models are of interest in their own right, but also may provide clues to long-term changes that are pertinent to the course of affective disorder. Study of the long-term consequences of cocaine administration may be relevant as a model of dysphoric mania and paranoid psychosis, and also may lead to novel treatment interventions for the current pandemic of cocaine use. These preclinical studies on cocaine administration in rodents will thus be integrated with clinical populations at these two levels. The tolerance paradigm involves anticonvulsant drug effects on kindled seizures, which we have found to be dependent on the temporal contingencies of drug administration relative to seizure occurrence. This model presents an opportunity to explore novel strategies to reinstate drug efficacy, and to determine how the nervous system adapts to chronic drug administration. This becomes increasingly important clinically, as psychiatric illness in particular, can have a life-long course making problems of drug tolerance, dependence, side effects, and refractoriness a grave concern.

1. Behavioral Sensitization:

Dr. Weiss has elucidated a novel one-day sensitization paradigm where animals are treated on day 1 with a high dose of cocaine (40 mg/kg, i.p.) or saline and tested on day 2 with a low dose of cocaine (10 mg/kg, i.p.). Animals pretreated with cocaine (compared with saline) in the context of the test cage show a greater response on day 2. Remarkably, animals treated with the same dose of cocaine on day 1, but in their home cages (instead of the test cage activity meter), show no difference in their behavior compared with the saline controls. Thus, cocaine sensitization, in this paradigm, is context-dependent and appears to be based on conditioned cues. This paradigm is highly robust and replicable, allowing dis-

crete pharmacological and neuroanatomical investigations. Moreover, this paradigm may be clinically relevant, as conditioned cues are now recognized as crucial variables in cocaine-related craving and relapse in humans.

A second sensitization paradigm consisting of multiple cocaine administrations (40 mg/kg, i.p. X 3 days) is also being studied in order to evaluate context-independent as well as context-dependent sensitization. The context-dependent sensitization produced in this paradigm is more long-lasting than in the 1 day paradigm, allowing for studies to elucidate the role of different brain structures or pharmacological manipulations in the expression of sensitization.

Dr. Weiss has found that there appear to be different substrates for the development vs. expression of cocaine sensitization. E.g. haloperidol, when administered in addition to cocaine on day 1, blocked the development of cocaine sensitization. However the same dose of haloperidol administered on day 2 in an attempt to block the expression of sensitization was without effect. Using more selective dopamine D1 or D2 antagonists, Dr. Fontana replicated and extended these findings and showed that both antagonists were capable of blocking development of sensitization but neither were effective on its expression. In contrast, clonidine and diazepam block both aspects of sensitization, and carbamazepine (given chronically or acutely) affects neither the development nor the expression of sensitization.

In collaboration with Drs. A. Pert and D. Fontana, Dr. Weiss has found that lesions of the amygdala (electrolytic or 6-OHDA) and lesions of the nucleus accumbens (6-OHDA) block the development of cocaine sensitization (while the day 1 cocaine-induced hyperactivity was unaffected). Lesions of the hippocampus or cerebellum were without effect in this paradigm. Lesions of the amygdala (electrolytic) do not block context dependent sensitization using the three day repeated cocaine administration paradigm [followed by a low dose (10 mg/kg) cocaine challenge on day 4]. This effect was replicated using animals with lesions of the amygdala tested first in the 1-day and subsequently in the 3-day paradigm. Thus, repeated exposure to cocaine renders the amygdala non-critical for context-dependent sensitization. Other substrates (e.g., the caudate) are being investigated regarding sensitization in the multiple exposure paradigm.

Rats treated with cocaine show cross sensitization to the local anesthetic procaine, but not to lidocaine or the stimulant caffeine. Others have shown that procaine (which is not a potent blocker of amine reuptake) is self-administered by monkeys like cocaine, but lidocaine is not self-administered, suggesting a parallelism between local anesthetic cross sensitization and self-administration. We have also found bi-directional context-dependent cross-sensitization between the NMDA antagonist MK-801 and cocaine.

2. Pharmacological kindling and its blockade by carbamazepine

Repeated administration of high doses of the local anesthetics lidocaine and cocaine result in the evolution of seizures to a previously subconvulsant dose. Following sufficient repetition of lidocaine-induced seizures, spontaneous seizures have been observed. A similar evolution might occur with cocaine; however, rats do not usually survive more than one or two bouts of cocaine-induced seizures. Thus, pharmacological kindling follows a sequential evolution of stages parallel to those observed in electrophysiological kindling: 1) development; 2)

completed; 3) spontaneous. These findings are of interest in relation to observations that cocaine can induce the evolution of panic attacks in man which also follows similar stages of evolution (Uhde). Initially, patients can administer cocaine for long periods of time without experiencing panic attacks. However, after many repetitions of cocaine-induced panic, spontaneous panic attacks may occur, even after the discontinuation of cocaine.

Dr. Weiss has observed that carbamazepine given chronically (orally) but not intermittently (i.p., once daily prior to cocaine or lidocaine) blocks the development of lidocaine- and cocaine-induced seizures and cocaine-induced lethality. This has led to the prediction that carbamazepine could demonstrate efficacy in preventing cocaine-induced panic attacks, or cocaine craving or self-administration in humans. A patent has been acquired for the latter. In collaboration with Dr. T. Aigner, cocaine self-administration was shown to diminish in rhesus monkeys co-treated with chronic oral carbamazepine. Carbamazepine was more effective at lower doses of cocaine per injection, which were associated with higher response rates. Remarkably, acute or repeated acute (intermittent) administration of carbamazepine is either ineffective (15 mg/kg) or exacerbates (50 mg/kg) cocaine kindling. A wide range of doses of carbamazepine (5-50 mg/kg) and time-points prior to cocaine (15 minutes to 5 hours) were evaluated to rule out differences in blood levels as a possible differential variable in the chronic vs. intermittent studies. The data suggest that different biological effects are being engendered by chronic compared with acute or intermittent carbamazepine administration. Since chronic administration of carbamazepine is required for its psychotropic effects in mania and depression, the local anesthetic seizure kindling may provide a unique model for exploring mechanisms of action of carbamazepine most closely related to its psychotropic efficacy.

Dr. Weiss has found that the following systems are not critical to the effects of carbamazepine on local anesthetic kindled seizures: serotonin (using PCPA-induced depletion), the peripheral-type benzodiazepine receptor, alpha-2-adrenergic receptors, and somatostatin mechanisms. The stress-related peptide CRH was demonstrated to markedly potentiate cocaine kindling and lethality, but only a non-selective reversal of carbamazepine's anticonvulsant effects was observed. Since carbamazepine is a tricyclic iminostilbene derivative, we evaluated the effects of another tricyclic antidepressant, desipramine (DMI), on cocaine kindling. DMI enhanced the cocaine-induced seizure development and lethality effects of cocaine, a finding which may have clinical significance since DMI is currently undergoing clinical trials for the treatment of cocaine abuse. Since chronic carbamazepine is like chronic caffeine in its ability to up-regulate adenosine A1 receptors, chronic and intermittent caffeine were tested for anticonvulsant potency against cocaine kindling. Chronic caffeine pretreatment (3 weeks before cocaine administration), but not chronic or intermittent co-treatment with caffeine, slowed cocaine kindling, suggesting that the up-regulation of adenosine systems might contribute to an anticonvulsant effect on local anesthetic kindling. Finally cholinergic mechanisms were evaluated using atropine, and physostigmine. Atropine inhibited cocaine kindling but enhanced lidocaine kindling; physostigmine had no effect on cocaine kindling and mildly inhibited lidocaine kindling. The effects of cholinergic manipulation on carbamazepine's anticonvulsant effects were also dependent upon the seizure model. Atropine and carbamazepine appeared to have additive inhibitory effects on cocaine kindling and lethality and physostigmine had no effect on carbamazepine's anticonvulsant effects in either model. The

reasons for the differential susceptibility of lidocaine and cocaine to cholinergic manipulation remain to be elucidated.

3. Pharmacological studies of amygdala kindling

Intermittent (repeated acute) carbamazepine, and its 10'11-epoxide metabolite are ineffective in preventing the development of amygdala kindled seizures, however, both drugs are highly effective on completed kindled seizures. This is the opposite of what is seen for local anesthetic seizures, in which carbamazepine is effective in the development but not completed phase of seizure evolution. Chronic carbamazepine is without effect on either electrical kindling development or completed seizures. The pharmacological dissociations observed with different phases of different types of kindled seizure evolution may have interesting clinical implications regarding treatment strategies over the course of illness. These data are also convergent with a large body of evidence indicating temporally based pharmacological dysjunctions: e.g., neuroleptics block development but not expression of conditioned sensitization; NMDA antagonists block the development but not maintenance of LTP.

Dr. Weiss has found that carbamazepine's acute anticonvulsant effects on amygdala-kindled seizures were blocked by the administration of Ro5-4864, an agonist at the peripheral-type benzodiazepine receptor, and the alpha-2-adrenergic antagonist yohimbine, but not by the central benzodiazepine antagonist Ro 15-1788, the inverse agonist ß-CCM; or the adenosine antagonists caffeine and theophylline.

Valproic acid was found to be an effective agent in slowing the development of amygdala kindled seizures, and against completed kindled seizures. Kindled animals that were rendered tolerant to the anticonvulsant effects of carbamazepine (See below) demonstrated cross tolerance to valproate, suggesting a potential common mechanism of action. A report that in vivo radiolabeled valproate was found to distribute exclusively in the olfactory bulbs led to the hypothesis that the peripheral-type benzodiazepine receptor (also distributed most densely in the olfactory bulbs of rats) might be important to the anticonvulsant effects of both carbamazepine and valproate. Lesions of the olfactory bulb were found to have no effect on the development of amygdala kindled seizures, the anticonvulsant responsivity to carbamazepine, valproate, or diazepam, or the development of tolerance to carbamazepine. Thus, although the peripheral-type benzodiazepine receptors seem to be involved in carbamazepine's anticonvulsant effects and tolerance development, the receptors in the olfactory bulb are not critical to this effect.

Dr. Weiss has discovered that anticonvulsant efficacy is determined, in part, by the occurrence of seizures. That is, when fully kindled rats were given time off from seizure stimulation (and anticonvulsant treatment), they lost their ability to respond to the anticonvulsant effects of carbamazepine. This occurred with time-off periods of 2 weeks, 1 week, and 4 days, but not 1 or 2 days. These data imply that an endogenous anticonvulsant principle is being induced by the seizures themselves, which facilitates anticonvulsant drug response. The time course of this effect will be used to help elucidate potential mechanisms of this phenomenon, e.g. increases in the anticonvulsant peptide TRH, changes in GABA receptors, etc.

4. Contingent Inefficacy and Contingent Tolerance

Dr. Weiss has discovered and replicated the observation of a new phenomenon termed contingent inefficacy. Treating animals with carbamazepine (15 mg/kg, i.p., prior to stimulation) during the development of electrical amygdala kindling seizure development (when it is ineffective) renders the animal subsequently resistant to carbamazepine's acute anticonvulsant effects at a time when the same dose is ordinarily highly efficacious (on completed kindled seizures). This resistance does not occur in animals given the same dose of carbamazepine during kindling development but not contingently (i.e., when carbamazepine is given after each amygdala-kindled stimulation instead of before), indicating that the inefficacy is not based on altered pharmacokinetics or mere prior exposure to the drug. This effect may be relevant to the development of anticonvulsant resistance in a high proportion of patients not controlled in the first several years of treatment of their epilepsy.

A similar phenomenon of contingent refractoriness was seen for valproate, although valproate did slow the development of the kindling process. However, when the animals did eventually develop kindled seizures, they had become partially or completely resistant to valproate's anticonvulsant effects (contingent refractoriness), whereas their cohorts treated with valproate-after each kindling stimulation were highly responsive to valproate at this phase of the kindling process. In both of these situations (contingent inefficacy for carbamazepine and contingent refractoriness for valproate) a reinstatement of anticonvulsant efficacy could be induced by a period of kindling the animals drug-free, or remarkably, by kindling the animals with drug administration after the kindled seizures. These data suggest that unpairing drug and kindling stimulation can reverse the contingent refractoriness.

Dr. Weiss has also demonstrated contingent tolerance to the acute anticonvulsant effects of carbamazepine on fully developed seizures. Carbamazepine treatment before each completed amygdala-kindled seizure (but not after) results in the development of tolerance to its anticonvulsant effect. This can be completely reversed by kindling the animals without drug or administering carbamazepine after each stimulation for a period of 5 or 7 days or longer. Contingent tolerance is not reversed by time off (no drug or stimulation) for ten days or three weeks or by injecting the animals with carbamazepine alone. Thus, it appears that the reversal of contingent tolerance and reinstatement of drug efficacy depends on the animals experiencing seizures in the absence of drug (i.e., an unpairing of drug and seizure). These data further support the importance of learning factors (temporal sequencing and pairing of drug with stimulation) rather than pharmacokinetic mechanisms in the development of tolerance.

Dr. Weiss has observed cross-tolerance between carbamazepine and PK-11195, a peripheral-type benzodiazepine receptor antagonist, but not between carbamazepine and diazepam, a central-type benzodiazepine agonist. Cross tolerance was also observed between carbamazepine and valproate, and to carbamazepine in animals experiencing contingent refractoriness to valproate (see above). Reversal of the contingent refractoriness to valproate also reinstated carbamazepine's anticonvulsant efficacy. In contrast to carbamazepine, tolerance to valproate was more difficult to induce and demonstrated large fluctuations under conditions of daily pairing of valproate (350 mg/kg) and electrical stimulation.

5. Changes in baseline seizure threshold in contingent tolerant animals: evidence for contingent compensatory mechanisms.

Assessment of seizure thresholds in animals that were responsive and tolerant to the anticonvulsant effects of carbamazepine has revealed the striking finding that seizure thresholds (measured in the medication-free state) decrease when contingent tolerance occurs and increase again when tolerance is reversed. This highly reproducible effect is supportive of Siegel's proposal of conditioned compensatory mechanisms developing as a mechanism for contingent tolerance phenomena. Since carbamazepine is an anticonvulsant, when tolerance to this effect becomes evident, it is associated with a compensatory proconvulsant adaptation. Moreover, animals similarly exposed to carbamazepine (but after the kindled seizures have occurred so that no tolerance develops) do not develop the change in seizure threshold.

Based on these data, Dr. Weiss predicted that the rate of tolerance development could be manipulated by the level of stimulation current. That is stimulation at a level just above the seizure threshold should result in slower tolerance development since a greater drop in threshold would be required to counter carbamazepine's threshold increasing effects. Stimulation at higher, suprathreshold currents might induce quick tolerance development since a small drop in threshold would be sufficient to counter carbamazepine's anticonvulsant effects. This prediction was borne out through direct testing of the same animals under both conditions of high vs. low stimulation current. The compensatory neurotransmitter and peptide systems that are responsible for the contingent changes in threshold are being investigated.

In addition to lower stimulation current tolerance development could also be slowed with the non-contingent presentation of the drug in addition to its contingent presentation (a situation most resembling the clinical state). That is, chronic co-treatment with the carbamazepine diet slowed tolerance development despite the continued acute pairing of the carbamazepine injection with the kindling stimulation. Higher doses, per se, of carbamazepine (i.e., 25 compared with 15 mg/kg) did not slow tolerance development, and a number of control studies demonstrated a lack of effect of the chronic carbamazepine diet on the acute dose-response curve for carbamazepine's anticonvulsant effects. Other attempts to pharmacologically manipulate the rate of tolerance development to carbamazepine using the NMDA receptor antagonist MK-801 and the calcium channel antagonist nimodipine were ineffective. Tolerance (loss of efficacy) can be a clinical problem when long-term drug use is required as is often necessary for treatment of psychiatric illness, epilepsy, and pain disorders. Thus, these data revealing novel variables to consider related to tolerance, and strategies of both slowing or reversing tolerance may be ultimately of considerable clinical importance.

6. Modulation of Kindled Seizure Thresholds

The kindling process is one in which convulsant responsivity is becoming altered over time, presumably as a result of changes in both excitatory and inhibitory processes. That is, while the nervous system is becoming progressively more excitable, corresponding attempts to limit or compensate for these changes may also be developing. This could explain why most clinical or experimental seizures do not develop into status epilepticus. Based on the findings of threshold adaptation in kindled animals paralleling drug tolerance, Dr. Weiss attempted to study whether seizure thresholds (presumably indicative of the summed excitatory and

inhibitory seizure mechanisms) could also be modified based on manipulation of the current intensity used for kindling stimulation. She found that with more intense electrical stimulation (i.e., higher currents) greater adaptive compensatory changes were also induced, as reflected in a higher seizure threshold.

Two groups of animals were kindled at either their afterdischarge threshold plus 50 μA (threshold), or at suprathreshold levels (800 μA). These groups developed kindled seizure at equal rates and showed no other differences during kindled seizure development. When these animals were fully kindled, their stimulation currents were switched so that the animals kindled at just above threshold received maximal stimulation for one week (800 μA) and the others received their threshold + 50μA as their stimulation current. Then these conditions were reversed to the previous (original) kindling conditions. When the rats that were kindled at 800 μA received threshold levels of stimulation, they did not show seizures or afterdischarges for several days, but slowly redeveloped seizures in an all or none fashion following about a week of further stimulation. The animals originally kindled at threshold, when raised to a current of 800 μA, continued to demonstrate their typical kindled seizures. However, when brought back to their original levels of stimulation (after one week at 800 μA), they did not have seizures or afterdischarges for a number of days. Again, several days were required to return to the original kindled state and this occurred in an all or none fashion.

These data suggest that adaptive mechanisms are continuously evolving during kindled seizure evolution and that they may develop to match the driving stimulus. Thus, they are susceptible to manipulation by alterations in the driving stimulus. In this case, kindling the animals for one week with a greater intensity stimulus rendered them less sensitive to their original kindling stimulus. This demonstration of differential modulation of endogenous adaptive mechanisms may have clinical implications regarding the evolution of greater endogenous compensatory mechanisms in epilepsy and affective illness following more severe episodes; treatments might ultimately be designed to optimally exploit these endogenous adaptive processes.

Unit on Behavioral Pharmacology (Agu Pert, Ph.D., Chief)

A. Conditioned and Unconditioned Effects of Psychomotor Stimulants

1. Conditioned components

The conditioning of cues associated with cocaine is an important component of the development and maintenance of sensitization. Dr. Pert has continued to use the simple and efficient one-day conditioning paradigm developed by Dr. Weiss to evaluate the variables regulating the acquisition and extinction of cocaine-induced conditioning. Three groups of rats are employed in this design. On day 1, the first group (PAIRED) is injected with a high dose of cocaine and placed in locomotor activity chambers. One hour following removal from the chambers, this group is injected with saline in the home cage. The second group (UNPAIRED) is injected with saline in the test chamber and a high dose of cocaine in the home cage. The third group (CONTROL) receives saline in both environments. On day 2, all animals are injected with a low dose of cocaine and placed in the activity chambers. The presence of conditioning is evidenced by the high activity scores of the PAIRED group on day 2 relative to the other two groups.

The following series of studies was conducted to elucidate some of the neuropharmacological and behavioral functions involved in the conditioned component of sensitization using the one-day design outlined above.

a. Extinction of cocaine-induced conditioning

The three groups of rats were treated as described above for seven days. For the next seven days, all animals were injected with saline (extinction for the PAIRED rats) and then on day-8, tested with a low dose of cocaine. The conditioned rats appeared relatively resistant to extinction, although by the seventh session there was no longer any difference among the three groups. Surprisingly, however, when the extinguished rats were challenged on day 15 with a low dose of cocaine, the conditioned effect was again fully expressed. Thus, it appears that it is possible to reinstate a seemingly extinguished conditioned cocaine effect with a small priming dose of cocaine.

b. Magnitude of the unconditioned stimulus (cocaine) as a determinant of conditioning

Rats were trained with various doses of cocaine on day 1 and then tested with 10 mg/kg of cocaine on day 2. The magnitude of the conditioned response appeared to be determined by the training dose, especially for stereotypy.

c. Centrally elicited conditioned cocaine effects

Rats implanted with cannulae guides aimed for the n. accumbens were conditioned for one day, as described above, and then challenged with cocaine in the n. accumbens on day 2. Centrally administered cocaine was also found to elicit a conditioned cocaine effect.

d. Ability of amphetamine to produce conditioning

Rats were trained as described above with amphetamine (2.5 mg/kg) instead of cocaine, and then challenged on day two with either saline or 0.5 mg/kg of amphetamine. Rats challenged with amphetamine showed only a very modest conditioned effect. Conditioning was more evident in rats challenged with saline. The relatively high dose of amphetamine on day 2 may have masked the conditioned effects. This variable needs further analysis.

e. Effect of glutamate blockade on cocaine-induced conditioning

Rats were pretreated with various doses of MK-801 (a glutamate blocker) or saline on day 1. Such blockade was found to prevent cocaine-induced conditioning when rats were tested with low doses of cocaine on day 2. It is possible that conditioned effect of cocaine involve corticifugal glutamatergic projections.

f. Effects of ECS on cocaine-induced conditioning

ECS was administered 1 hour prior to conditioning, immediately following conditioning, one hour following conditioning, or one hour prior to testing on day 2. Only ECS administered immediately following training was effective in preventing conditioning. These findings clearly indicate that cocaine-induced conditioning involves associative processes which appear to consolidate in at least one hour.

2. Unconditioned components

Attempts were made to elucidate the neurochemical mechanisms underlying the behavioral sensitization induced by chronic amphetamine. Rats were treated for seven days with 3 mg/kg amphetamine and then challenged on day eight or 11 with a lower dose. Amphetamine applied through microdialysis probes to the terminal fields of the dopamine pathways elicited a dose-dependent increase in dopamine overflow. No differences were found between animals treated chronically with

amphetamine or saline either one or four days following termination of treatment. A systemic challenge of 1 mg/kg also did not reveal a different in the dopamine response of the striatum, while a challenge with 0.25 mg/kg one day following treatment produced a more prolonged increase in striatal dopamine overflow in the chronic amphetamine group. While it is possible that increased responsivity of the dopamine system to amphetamine may underlie some aspects of behavioral sensitization, pharmacokinetic factors must also be considered.

B. Actions of psychoactive drugs on brain neurochemistry

1. Differential effects of amphetamine and cocaine in the amygdala and striatum

There are reasons to suspect that dopamine (DA) transporters are not the same in all regions of rat brain. Various concentrations of amphetamine and cocaine were introduced into the striatum or amygdala via microdialysis probes implanted in either structure. The dialysates were assayed for dopamine and dopamine metabolites. Infusions of cocaine into the striatum produced a concentration-dependent increase in DA overflow while infusions into the amygdala were ineffective. The striatum also appeared to be more sensitive to amphetamine. These findings suggest that there are fundamental differences in DA transporter functions in these two brain regions.

2. Effects of cocaine, morphine, and MK-801 on striatal acetylcholine

Systemic injections of low doses of cocaine produced significant elevations in striatal acetylcholine while a high dose (40 ng/kg) produced a smaller effect. Procaine failed to alter striatal acetylcholine. The elevations in acetylcholine are presumably mediated through a D_1 DA receptor action. Low doses of morphine also elevated striatal ACh while low doses of MK-801 produced a significant decrease. The mechanisms by which the latter two drugs exert their actions on striatal ACh are presently being explored.

3. Effects of cocaine on brain norepinephrine.

In vivo microdialysis was used to characterize the effects of focal and systemic cocaine on extracellular NE in the hippocampus and frontal cortex. Applications of cocaine to the hippocampus via dialysis probes produced a concentration-dependent increase in extracellular NE. In the frontal cortex, on the other hand, only the highest concentration of cocaine (100 μm) produced an increase in extracellular NE. Systemic administration of cocaine produced no effect on extracellular NE in either structure. The differential effects of cocaine in the two regions may suggest differences in the affinity of cocaine for the NE uptake sites.

4. Alterations in raphe and frontal cortex serotonin overflow by fluoxetine

Fluoxetine is an antidepressant that is a potent inhibitor of serotonin (5-HT) reuptake. The purpose of this study was to characterize the effects of this uptake inhibitor on 5-HT overflow measured simultaneously in the raphe and frontal cortex. Fluoxetine applied focally to the frontal cortex or raphe region increased extracellular 5-HT. A concurrent decrease of 20% in 5-HT occurred in each normally perfused region following focally applied fluoxetine at the other site. Systemic fluoxetine increased 5-HT in the raphe but decreased it in the frontal cortex. The net effect of fluoxetine appears to be determined predominantly by increased 5-HT in the somatodendritic region, which inhibits raphe firing, resulting in a decrease in cortical release.

5. Alterations in mesolimbic dopamine overflow by opioids and electrical brain stimulation

Electrical stimulation of the VTA as well as injections of enkephalin into this region produced increases in the n. accumbens DA and locomotor activity. These two effects appeared to be dissociated.

c. Neurobiology of non-competitive NMDA antagonists

Injections of the GABA agonist muscimol into the globus pallidus contralateral to a 6-OHDA medial forebrain bundle lesion inhibited ipsilateral rotational behavior in rats injected with both amphetamine and MK-801. These findings suggest that both stimulants are exerting their motoric effects by inhibition of GABA transmission in the globus pallidus. Injections of low doses of MK-801 to freely moving rats decreased acetylcholine levels in the striatum at the same time increasing locomotor output. These findings support the possibility that acetylcholine interneurons in the striatum may be under glutamatergic regulation.

Unit on Neurochemistry (Mike Clark, Ph.D.)

Dr. Clark's work focuses on the role of immediate-early genes in kindling, receptor alterations with kindling and contingent tolerance, the mechanism of action of carbamazepine (with particular emphasis on adenosinergic mechanisms), and new approaches to the neurobiology of poly-ADP-ribosylation. Previous summaries have documented the remarkable spatio-temporal evolution of the induction of the proto-oncogene c-fos with amygdala kindling evolution.

Dr. Clark has now begun autoradiographic studies of various receptors to investigate the biochemical mechanism(s) of associative or contingent tolerance to carbamazepine (CBZ). The following receptor systems were assayed in amygdala kindled and control animals as well as in rats that were made tolerant to the anticonvulsant effects of CBZ and appropriate control rats: (1) Glutamate NMDA subtype with [^{3}H]MK-801, (2) $GABA_A$ with [^{3}H]muscimol and [^{35}S]TBPS, (3) Central--type benzodiazepine with [^{3}H]Ro 15-1788, (4) Peripheral-type benzodiazepine with [^{3}H]Ro 5-4864, and (5) Adenosine A_1 and A_{2a} subtypes with [^{3}H]cyclohexyladenosine and [^{3}H]CGS-21680, respectively.

The findings have demonstrated that $GABA_A$ receptors and central-type benzodiazepine receptors show increased binding in the dentate gyrus of the hippocampus with electrical kindling of the amygdala. These findings have been extended with the demonstration that [^{35}S]TBPS binding is also increased in the dentate gyrus with kindling. No changes in striatal adenosine A_{2a} receptor binding were observed. Likewise, no changes in the hippocampus were observed for adenosine A_1 or NMDA receptors. [^{3}H]Ro 5-4864 was not a suitable ligand for autoradiographic analysis of peripheral-type benzodiazepine receptors.

Interestingly, in rats made tolerant to CBZ (contingent tolerance), $GABA_A$ receptor binding ([^{3}H]muscimol) was selectively decreased toward control levels as compared to non-tolerant kindled rats, while neither central-type benzodiazepine receptor nor [^{35}S]TBPS binding differed between tolerant and non-tolerant rats. This finding suggests that the mechanism of contingent tolerance to CBZ involves a decrease in $GABA_A$ receptor function in the dentate gyrus without involvement of the benzodiazepine or TBPS sites. This demonstration of a biochemical correlate

of contingent tolerance to CBZ in the amygdala kindling paradigm is one of the first observations of a contingent drug effect and opens up new avenues of investigation of the biochemical mechanisms involved in these associative changes.

In other studies of peripheral-type benzodiazepine receptor mechanisms, lidocaine, carbamazepine and valproic acid were tested as inhibitors (dose-responses) of agonist [^{3}H]Ro 5-4864 and antagonist [^{3}H]PK 11195 binding in olfactory bulb homogenates. It was found that each of these compounds had greater affinity for the agonist subsite (Ro 5-4864) of the PBR. These data confirm other investigators works that suggest different conformational states of the PBR exist (possibly with different functional consequences).

[^{3}H]PK 11195 binding in the olfactory bulb revealed increased density and decreased affinity of PBR in amygdala kindled rats receiving multiple stage 5 seizures compared to sham control rats, 24 hr after the last seizure. Dr. Clark plans to test PBR binding in other brain regions (e.g., hippocampus, cerebral cortex, striatum, cerebellum) to determine whether kindling alters this system in selective brain regions.

It was recently reported by others that the PBR are involved in steroidogenesis by increasing cholesterol transport into mitochondria. Dr. Clark's preliminary data suggest that carbamazepine also increases cholesterol uptake by rat brain mitochondria with resultant increased cholesterol metabolism. Since carbamazepine binds to PBR, it is hypothesized that carbamazepine does increase steroid synthesis in rat brain. This may be an important part of the CNS effects of carbamazepine.

Studies on identifying the hypothesized central adenosine A_3 receptor show that a small component of apparently A_1 binding is displaced by nicotinamide adenine dinucleotide (NAD) which was previously shown by others not to bind at adenosine receptors. [^{3}H]NAD binding was shown to be stereospecific by autoradiographic analysis. [^{3}H]NAD binding is partially inhibited by adenosine agonists and by carbamazepine. These preliminary results hint at the possibility of an unidentified adenosine receptor which also recognizes NAD.

These studies have led to the discovery of an endogenous ADP-ribosylation caused by a sulfonated dye (Fast Green FCF) that was found to differentially alter agonist binding to adenosine A_1 and A_2 receptors. Fast Green FCF stimulated adenosine A_1 binding modestly (up to 20% at 1mM), while it dose-dependently inhibited adenosine A_2 binding (up to 42% at 1 mM). Moreover, Fast Green FCF dose-dependently inhibited PBR binding almost completely but had no effect on binding at the central-type benzodiazepine receptor. The effect of Fast Green FCF on PBR binding was potentiated by $MgCl_2$. It was found that Fast Green FCF dose-dependently stimulated the transfer of ADP-ribose from NAD to brain proteins (ADP-ribosylation). This effect of the dye is markedly potentiated by $MgCl_2$. Protein gel electrophoresis of ADP-ribosylated proteins revealed two major bands with molecular weights of about 117 kDa and 10 kDa. The stimulatory effects of Fast Green FCF on ADP-ribosylation was highly localized to the purified nuclear fraction. Enzymatic digestion of the product coupled with HPLC analysis conclusively showed that Fast Green FCF stimulated the nuclear enzyme poly(ADP-ribose) polymerase to produce polymers of poly(ADP-ribose) from NAD. Since this enzyme is activated by drugs and procedures (e.g., radiation) that cause DNA damage, it was tested whether Fast

Green FCF caused DNA damage as a potential mechanism for its stimulation of poly(ADP-ribosylation). However, the dye did not appear to elicit DNA damage. The mechanism of the dye's stimulation may prove important to the understanding of poly(ADP-ribose) polymerase activation and the potential involvement of poly(ADP-ribose) in transcription. The mechanism of dye-induced poly(ADP-ribosylation) requires further investigation.

Unit on Neurochemistry - (Jeffrey Rosen, Ph.D)

A major focus of Dr. Jeffrey Rosen's lab has been the investigation of mRNA expression of neuropeptides associated with amygdala-kindled seizures. Following the mapping of the spatio-temporal evolution of c-fos mRNA expression during amygdala kindling by Clark and associates, Dr. Rosen has mapped alterations in the expression of several neuropeptide mRNAs during kindling. Clark et al. showed increases in c-fos mRNA in the pyriform and entorhinal cortices, hippocampus, and dentate gyrus following an afterdischarge or seizure at different stages of kindling, suggesting a spatio-temporal evolution of c-fos activation with the development of kindling. Because FOS may act as a transcription factor for peptide genes, expression of mRNAs of enkephalin, dynorphin and thyrotropin releasing hormone (TRH) following kindling was investigated by in situ hybridization.

Enkephalin mRNA expression was increased ipsilateral to the stimulated amygdala in the entorhinal cortex 24 hours after a stage 1 seizures. However, 24 hours after a stage 5 seizure, bilateral increases were found in the entorhinal and pyriform cortices. Increases were long-lasting and still evident in the pyriform cortex two weeks after a stage 5 seizure. In contrast, the levels of dynorphin mRNA were decreased in the dentate gyrus 24 hours after a stage 1 or 5 seizure. Changes in TRH mRNA with kindled seizure evolution were the most interesting because basal levels were almost undetectable. Following a seizure, however, the mRNA levels rose dramatically and followed the anatomical pattern of increases seen with c-fos mRNA. Similar to that observed with c-fos, TRH mRNA levels in the majority of rats were increased ipsilaterally to the stimulation in the pyriform and entorhinal cortices after stage 1 seizures, however, there was also a second pattern where TRH mRNA levels did not rise in the pyriform and entorhinal cortices, but were increased bilaterally in the dentate gyrus. Twenty-four hours after a stage 5 seizure, increases were seen bilaterally in all these regions plus the perirhinal cortex. The patterns of TRH mRNA induction closely followed those of c-fos with kindled seizure development. Thus, some neuropeptides such as TRH, which may be target genes for the c-fos dependent transcription factor, display a spatio-temporal pattern of activation similar to c-fos, suggesting a function association.

To further explore the relationship between the c-fos and TRH mRNA patterns following kindled seizures, double labelling techniques were used to examine whether members of the Fos family of transcription factors co-localized with TRH mRNA in regions important for kindling. Brain sections of rats sacrificed 5 hours after a stage 5 seizures were sequentially processed for in situ hybridization of TRH mRNA followed by immunohistochemistry for Fos and Fos-related antigens (Fras) with an antibody that recognizes a common epitope of Fos and Fras. A very high percentage of the Fos labelled cells also had TRH mRNA labelling. The percent of Fos labelled cells with TRH mRNA labelling in the pyriform cortex was 62%, in the entorhinal was 70% and in the perirhinal was 80%. Intense TRH mRNA and Fos-like

immunohistochemical labelling were found in the dentate gyrus, but were too dense to measure. Interestingly, in the reticular nucleus of the thalamus and several nuclei in the hypothalamus where there are high basal levels of TRH mRNA, Fos and TRH mRNA were not co-localized. These results demonstrate a high correlation between the induction of Fos and TRH following kindled seizures, suggesting that Fos or Fras may act as transcription factors for TRH in limbic areas important for kindling.

In a study related to the alterations of neuropeptide mRNAs following kindling, Dr. Rosen explored the question of whether the transient changes seen after a single stage 5 seizure could become more long-lasting following 20 stage 5 seizures. The levels of enkephalin, dynorphin and TRH mRNAs increased with more seizures, however, they were no more long-lasting than following a single seizure. Only alterations of enkephalin mRNA levels in the pyriform cortex were still evident two weeks following 20 seizures, the same as after one seizure. The alterations in other neuropeptides were also not different. This suggests that the neuropeptides play a transitory role in kindling and may be an intermediate step in a sequela of alterations leading to more long-lasting changes with kindling.

Another interest of the lab is biochemical and molecular changes associated with cocaine sensitization. Others have shown that c-fos mRNA and its Fos protein product are increased in cells in the striatum with a single injection of cocaine or amphetamine. Studies carried out in collaboration with Dr. Michael Iadarola of the NIDR, have shown that with repetitive, intermittent administration (either four injections once every two hours or one injection per day for several days) of cocaine, both c-fos mRNA and Fos protein levels were decreased by about 50% compared to a single cocaine injection. At the same time the rats which received multiple injections showed behavioral sensitization, displaying more behavioral stereotypies than the rats that had a single injection. Whether these changes in Fos are sensitive to environmental, contextual cues which have been shown to control behavioral sensitization are beginning to be explored.

Context conditioning to a fearful stimulus has been shown to sensitize rats to acoustic stimuli. In collaboration with Drs. Michael Davis and Eric Nestler at Yale University, Dr. Rosen has shown that the induction of c-fos mRNA in the amygdala is very sensitive to contextual fear conditioning. When rats were reintroduced to an environment that they had previously been given footshocks, they showed increased startle responses. In addition, reintroduction also c-fos mRNA dramatically increased in the amygdala. This is the first study to show that c-fos is increased following unconditioned and conditioned fear in the amygdala, a nucleus known to be crucial for fear conditioning and possibly sensitization. The possibility of different kinds of sensitization (cocaine-, fear- and kindling-induced) using some of the same neuroanatomical substrates is intriguing.

Unit on Molecular Neurobiology (De-Maw Chuang, Ph.D., Chief)

Members of the Unit on Molecular Neurobiology continue to study the regulation of neurotransmitter receptor expression, neurotransmitter induction of second and third messengers, neurotoxicity, and neuroprotection. This group is particularly interested in how each of these phenomena relate to psychotropic medicines, especially lithium and carbamazepine (CBZ). Investigation of these general questions has been approached by detailed study of muscarinic acetylcholine receptors

(mAChRs), serotonin (5-HT) receptors, β-adrenergic receptors (βARs), δ-opioid receptors, glutamate receptors, and receptors for neuropeptides. Special focuses are on the interrelationship between cytoskeleton and receptor modulation, neurotoxicity induced by CBZ and glutamate, and the mechanism by which the density and affinity of each receptor is regulated. Both in vitro systems, including cultured rat cerebellar neurons and astrocytes, and in vivo systems, primarily chronic administration of psychotropic agents to rodents, have been employed in the work of the Unit.

mAChRs mediate many of the actions of acetylcholine in the CNS and are thought to be relevant to some pathological states including cognition and memory dysfunction, Alzheimer's disease and, perhaps, affective disorders. Drs. Fumihiko Fukamauchi (a Visiting Fellow), Christopher Hough, and Chuang have used cerebellar granule cells (CGCs) as a model to elucidate mAChR adaptation in response to known mAChR ligands, drugs affecting microtubules, lithium, and a novel neuropeptide endothelin. CGCs are prepared from neonatal rats to allow differentiation in culture into glutamatergic neurons with a purity of greater than 90% of the total population. The group has documented that in vitro cerebellar neurons express mRNA for m_2- and m_3-mAChRs; these receptors are coupled to adenylate cyclase and phospholipase C, respectively. Receptor binding studies using subtype-specific antagonists revealed that the m_3-mAChR is the predominant receptor subtype, comprising approximately 80% of the total mAChR sites. They have shown that stimulation of CGCs with the mAChR agonist carbachol induces time- and concentration-dependent decreases of the steady state level of m_2- and m_3-mAChR mRNA. The time courses of down-regulation of m_2- and m_3-mAChR mRNAs are distinct, however. This mRNA down-regulation contributes to the early phase of agonist-induced loss of mAChR binding sites and is reversible upon the removal of the prestimulating agonist. Preliminary results indicate that the decrease of mRNA level is the result of decreased transcription rather than enhanced mRNA degradation. Carbachol-induced down-regulation of m_2- and m_3-mAChR mRNAs is blocked by the nonselective antagonist atropine, while the subtype-selective antagonists AF-DX116 and 4-DAMP block the down-regulation of their corresponding mAChR mRNAs.

In order to gain insight into the mechanisms involved in the modulation of mAChR gene expression, the group has investigated the effects of mAChR antagonists on their receptor mRNA levels in CGCs. Atropine induces a time- and concentration-dependent elevation of m_2- and m_3-receptor mRNAs and a concurrent increase in the density of mAChR binding sites. This mRNA up-regulation appears to involve an increase in the transcriptional rate as measured by a nuclear RNA runoff experiment. After atropine wash-out, the up-regulated mAChR mRNA and mAChR binding sites return to the untreated level, again suggesting a major role for mAChR mRNA in determining the density of the receptor protein. Treatment of neurons with the receptor subtype-selective antagonists AF-DX116 and 4-DAMP also results in an up-regulation of m_2- and m_3-mAChR mRNA, respectively. Several lines of evidence strongly suggest that cAMP may be involved in negative regulation of m_2- but not m_3-mAChR mRNA.

The cytoskeleton has been implicated in the pathogenesis of Alzheimer's disease and is a possible target for antidepressant drugs. Dr. Fukamauchi and his co-workers have explored a possible role of microtubules in the regulation of mAChR mRNA. Colchicine, a disrupter of microtubule structure, elicits a time- and concentration-dependent increase of m_2-mAChR mRNA but a concomitant decrease of

m_3-mAChR mRNA in CGCs. Colchicine treatment also induces a concurrent decrease in total mAChR binding sites, m_3-mAChR-mediated phosphoinositide turnover, and the cAMP content, but it produces an increase in the mRNA level of the proto-oncogene c-fos. These effects of colchicine are reversed by taxol, a microtubule-stabilizing agent. Moreover, β-lumicolchicine, an inactive colchicine analog, does not elicit any of the changes. Taken together, these findings suggest a pivotal role for the cytoskeleton in the hemostasis of m_2- and m_3-receptor mRNA.

In an attempt to shed light on the action and side effects of the clinical use of lithium, the team has investigated this drug's long-term effects on mAChR gene expression and signal transduction in CGCs. At a therapeutically relevant concentration range (0.5-2.0 mM), lithium chloride, after treatment for seven days, induces an 80-100% increase in m_3-mAChR mRNA, but a decrease in m_2-mAChR mRNA in CGCs. This effect is associated with a slight but significant increase in total mAChR binding sites and carbachol-induced phosphoinositide hydrolysis. c-Fos mRNA and the outgrowth of neurites are also markedly enhanced, indicating a trophic action of lithium. At higher concentrations ($\geq$ 5 mM), this drug induces a drastic reduction in m_2- and m_3-mRNA, c-fos mRNA, and a deterioration of neuronal morphology, presumably due to lithium-induced neurotoxicity. Since the neurotrophic action of lithium occurs at a clinically relevant concentration and requires long-term treatment, this effect may be related to its efficacy in the treatment of bipolar depressive illness.

Dr. Chuang and his group have conducted a series of pioneering studies demonstrating that endothelin-1 (ET) is a novel neuropeptide. In their recent study, they investigated the effects of ET in the expression of mAChR mRNA in CGCs. ET was found to induce a relatively rapid up-regulation of both m_2- and m_3-receptor mRNA. This up-regulation is preceded by an increase of c-fos mRNA and is abolished when c-fos mRNA induction is prevented by 2-aminopurine or cycloheximide. Suppression of ET-induced phosphoinositide breakdown by a brief pre-exposure to a phorbol ester (which activates protein kinase C), also markedly inhibits the ET-induced up-regulation of mAChR mRNAs. Their findings suggest that this novel neuropeptide positively regulates transcription through its action on the inositol trisphosphate/diacylglycerol signaling pathway.

Dr. Jotaro Akiyoshi (a Visiting Fellow) in Dr. Chuang's group has expanded our understanding of the control of neurotransmitter receptor gene expression by exploring the effects of 5-HT receptor agonists, antagonists, and psychotropic drugs on the regulation of serotonin receptors. 5-HT, by interacting with multiple 5-HT receptors, induces an array of neurophysiological responses, including sleep, cognition, mood control and sexual behaviors. The group has demonstrated that CGCs express 5-HT_2 receptor subtypes coupled to phospholipase C through a pertussis toxin-sensitive mechanism. Prestimulation of these neurons with 5-HT or DOI (2,5-dimethoxy-4-iodophenylisopropylamine), a putative 5-HT_2 receptor agonist, results in a time-dependent desensitization of 5-HT-induced phosphoinositide response. Interestingly, ^{3}H-ketanserin binding to 5-HT_2 receptors is increased following the onset of desensitization induced by 5-HT or DOI. This augmentation persists for at least 24 hours and is associated with an increase in the B_{max} and K_d of ^{3}H-ketanserin binding to 5-HT_2 receptors. 5-HT_2 receptor mRNA detected by Northern blot hybridization is also increased by 60-100% in parallel with the up-regulation of 5-HT_2 receptor binding sites induced by 5-HT or DOI, while the alpha subunits of Gi and Go proteins remain unchanged.

In a related series of experiments, Dr. Akiyoshi and colleagues examined the effects of two 5-HT_2 receptor antagonists, mianserin and ketanserin, on 5-HT_2 receptor-mediated signal transduction. Both antagonists elicit a time-dependent desensitization of 5-HT-induced phosphoinositide turnover and a concomitant decrease of ^{3}H-ketanserin binding to 5-HT_2 receptors. These effects of antagonists are fully manifest approximately two hours after exposure with a partial reversal thereafter. Preliminary results show that mianserin-induced desensitization and receptor down-regulation are accompanied by a decrease of the 5-HT_2 receptor mRNA levels. This effect may be clinically relevant, as mianserin is an antidepressant which has been shown to induce a similar down-regulation of 5-HT_2 receptor binding sites and 5-HT-induced effector responses in vivo. These results also demonstrate, for the first time, that the expression of 5-HT_2 receptors is regulated by their agonists and antagonists in an unusual manner, namely, up-regulation by agonists, and down-regulation by antagonists. Dr. Akiyoshi has also established that mRNA of 5-HT_{1A} receptors is present in CGCs; he has begun to look into potential cross-regulation between 5-HT_{1A} and 5-HT_2 receptors in these neurons. In addition, he has initiated an in vivo study of the effects of various chronic treatments in rats on the expression of various 5-HT receptor subtypes and their second messenger production. These pharmacological manipulations include chronic administration of imipramine, desipramine, amitriptyline, fluoxetine, buspirone, lithium and carbamazepine, as well as repeated electroconvulsive shock treatment.

The group has also used CGCs as a model to study the mechanisms of action of excitotoxins and neuroprotectants. Drs. Xiao-Ming Gao (a Visiting Associate) and Chuang have recently documented that carbamazepine (CBZ), an anticonvulsant now widely used in a number of psychiatric disorders, is neurotoxic for CGCs. The toxic effect is evident only after several days and can be demonstrated with both morphological and biochemical methods. The neurotoxicity occurs in the concentration range of 20-100 μM; since the range overlaps but largely exceeds the therapeutic plasma level of CBZ, the toxicity may be related to the side effects of CBZ; i.e., teratogenesis and overdosage effects such as abnormal movements, coma, and seizures. In a related study, Drs. Chuang and Gao, in collaboration with Dr. Steven M. Paul (Section on Molecular Pharmacology, CNB, NIMH), have demonstrated that glutamate also elicits a delayed toxicity in CGCs through activation of N-methyl-D-aspartate (NMDA) receptors.

Surprisingly, the neurotoxic effects of both CBZ and glutamate are completely blocked by NMDA which, by itself, is not neurotoxic to CGCs. Other excitatory amino receptor agonists such as quisqualate, kainate and ibotenate produce either weak or no neuroprotection. The neuroprotective action of NMDA is relatively specific for CBZ and glutamate; the neurotoxicity of lithium, veratridine (a voltage sensitive Ca^{2+} channel activator), and colchicine is not affected by NMDA. Several lines of evidence argue against the possibility that the neuroprotection is due to NMDA-elicited desensitization of NMDA receptors. The precise mechanisms underlying this neuroprotective effect are being investigated. CBZ-induced neurotoxicity is not sensitive to the NMDA receptor antagonists, MK-801 and aminophosphovalerate, or the peripheral benzodiazepine receptor ligands, PK-11195 and Ro 5-4864. However, CBZ can displace ^{3}H-MK-801 binding to NMDA receptors with a modest affinity (IC_{50}=28 μM). Furthermore, prolonged treatment with CBZ results in an up-regulation of NMDA receptor binding sites, NMDA receptor-mediated phosphoinosi-

tide turnover and the release of preloaded ^{3}H-D-aspartate. These results suggest that CBZ toxicity in CGCs is mediated by indirect allosteric interaction of CBZ with NMDA receptors. To better understand the CBZ neurotoxicity and NMDA neuroprotection Dr. Christopher Hough, in collaboration with Dr. Michael Rogawski (Medical Neurology Branch, NINDS), has begun to characterize the NMDA receptor-mediated Ca^{2+} channel in single CGC preloaded with fura-2 and to examine the influence of CBZ on this event.

In another study, Dr. Chuang and Peter Leeds have observed neurotoxic effects induced by nonselective protein kinase antagonists (H_7 and staurosporine) and selective tyrosine kinase inhibitors (genistein and lavendustin A) in CGCs. The neurotoxic effects of these agents are not blocked by NMDA or a phorbol ester, with the exception that a phorbol ester partially blocks the neurotoxic effects of staurosporine. In a collaborative effort, Drs. Chuang and Roichi Ishitani's group (Josai University, Japan) have shown that THA (9-aminotetrahydroacridine), similar to NMDA, has a neurotrophic effect that promotes the survival of CGCs cultured in the presence of low (15 mM) KCl. However, unlike NMDA, THA selectively increases the activity of m_3-mAChR-mediated phosphoinositide turnover and the density of mAChR binding sites. This finding may provide a new avenue for approaching the mechanisms of action of THA and related drugs in the treatment of Alzheimer's patients.

In light of the implication that β-adrenergic receptors (βARs) are involved in the actions of antidepressant drugs, Drs. Hough and Chuang have used rat C_6 glioma cells as a model to unravel the molecular mechanisms underlying gene regulation of βARs. They have previously determined that β_1- and β_2-AR mRNAs are differentially modulated in response to stimulation with the βAR agonist isoproterenol. Although β_2-AR mRNA shows a monophasic down-regulation by isoproterenol, β_1-AR mRNA is initially up-regulated and then down-regulated. Like carbachol-induced down-regulation of mAChR mRNA in CGCs, this isoproterenol-induced βAR mRNA down-regulation is not due to a decreased stability of these receptor mRNAs. Moreover, blockade of protein synthesis by cycloheximide markedly stabilizes β_1- and β_2- AR mRNA, suggesting either that protein synthesis is necessary for mRNA degradation or the involvement of a highly labile protein in this degradation.

While cAMP is likely to participate in this agonist-induced mRNA regulation, they have obtained evidence that other mechanisms are also involved in the control of βAR expression, as exemplified by the effects of βAR antagonists, microtubule-affecting drugs and carbamazepine (CBZ). βAR antagonists such as propranolol, alprenolol, and subtype-specific blockers (betaxolol and ICI 118,551) all induce changes in β_1- and β_2-receptor mRNA in a complex manner in C_6 glioma cells. These antagonist effects are stereospecific, subtype-specific, and appear to be independent of cAMP production. In another study, disruption of microtubule structures by colchicine also elicits distinct changes in β_1- and β_2-AR mRNA levels. β_1-AR mRNA is initially increased before it is down-regulated to about 50% of control. In contrast, β_2-AR mRNA shows a delayed up-regulation by colchicine. When C_6 glioma cells are pretreated with colchicine prior to isoproterenol stimulation, isoproterenol down-regulation of β_1-AR mRNA is not just completely blocked, but actually increases to double the untreated level. The down-regulation of β_2-AR mRNA, on the other hand, is enhanced. These results affirm a prominent role for the cytoskeleton in controlling the steady state pool of neurotransmitter receptor mRNA.

In collaboration with Dr. William Z. Potter's section (ETB, NIMH), Drs. Hough and Chuang have found that the CBZ-induced increase in the density of BAR binding sites is associated with an up-regulation of β_2-AR mRNA. Preliminary data show that the peripheral benzodiazepine receptor ligands PK11195 and Ro 5-4864 also induce a similar increase of β_2-AR mRNA. These observations raise the possibility that CBZ acts on mitochondrial peripheral benzodiazepine receptors to effect the transport of cholesterol and biosynthesis of pregnenolone and neurosteroids. The latter may then serve as a transcriptional modulator for β_2-AR expression. In a related study, they found that down-regulation of βAR in C_6 glioma cells by long-term desipramine treatment is accompanied by a decrease of β_1- but not β_2-AR mRNA, as reported for the in vivo effect of this antidepressant. Since cultured C_6 glioma cells are devoid of noradrenergic innervation, this model system provides a new avenue for exploring a direct action of antidepressants and related drugs on βAR expression in target cells. Recently, the team has detected the mRNAs for 5-HT_{1A} and 5-HT_2 receptors in C_6 glioma cells. Studies on possible cross-regulation between 5-HT receptors and βARs are in progress.

Connexin43 is one of the protein isoforms that compose the gap junction which is a route to rapid intercellular communication among astrocytes and between astrocytes and neurons. Connexin43 is expressed constitutively in C_6 glioma cells. Drs. Hough and Russell L. Margolis (a PRAT Fellow) in the group have found that isoproterenol elicits a 3- to 4-fold increase in connexin43 mRNA in a time- and cell density-dependent manner. The increase in connexin43 mRNA is blocked by βAR antagonists and mimicked by cAMP analogues. Moreover the presence of colchicine markedly attenuates the up-regulation of connexin43 mRNA. The molecular details of this effect of isoproterenol are being investigated, along with the hypothesis that pharmacological manipulations of connexin43 quantity may alter the passage of Ca^{2+} among glial cells and between glia and neurons.

Dr. Margolis has also examined the effects of electroconvulsive and kindled seizures, as well as psychotrophic drug effects on connexin43. In collaboration with Drs. Weiss and Hough in the Branch, he has approached the problem of CBZ pharmacology by looking at the effect of chronic CBZ and lithium treatment on the mRNA of connexin43, which has been reported to be up-regulated in human seizure disorders. Preliminary results suggest that lithium, but not CBZ, tends to reduce connexin43 mRNA levels in most brain regions; however, in the hippocampus, CBZ, but not lithium, has this effect. To pursue these findings in more detail, Drs. Margolis and Hough found that CBZ, like isoproterenol, up-regulates connexin43 mRNA in C_6 glioma cells. Further studies are underway to better understand the action of CBZ in vitro. In collaboration with Dr. Jim Olds (Lab. of Molecular & Cellular Neurobiology, NINDS), Dr. Margolis has found that translocation of a third messenger, protein kinase C, to the cytoplasmic membrane, a putative measure of the kinase activity is decreased in the substantia nigra of rats contingently tolerant to CBZ. A project to confirm and expand upon this conclusion is currently underway. Dr. Margolis has also found that c-fos mRNA is transiently expressed after one seizure, but is down-regulated following seven seizures. Connexin43 mRNA also appears to be down-regulated after seven seizures. The study of other genes, and a full replication of the preliminary results, are currently in progress. Given recent reports in the literature that kindling decreases the number of neuronal cells in the hilar polymorphonuclear region of the hippocampus, Dr. Margolis has used Northern blot analysis to look for mRNA changes indicative of

cell death (apoptosis) after kindling. The mRNA for TRPM2, a species of protein known to increase dramatically in certain forms of apoptotic cell death, is not substantially altered by kindling; the regulation of other types of mRNA implicated in apoptosis is currently under investigation.

Drs. Wan-Wan Lin and Chuang have expanded their studies on neuropeptide receptors and purinoceptors in C_6 glioma cells and cerebellar astrocytes. Because increasing evidence indicates that glial cells are a target for endothelin, they have characterized the effector responses mediated by endothelin-1 (ET) receptors in C_6 glioma. Stimulation with ET induces phosphoinositide hydrolysis to generate inositol trisphosphate and cause Ca^{2+} influx through a receptor-gated channel. Both events are dependent on external Ca^{2+} and sensitive to inorganic blockers of Ca^{2+} channels (Cd^{2+}, La^{3+} and Mn^{2+}). The former response is mediated by a pertussis toxin-sensitive G protein, leading to intracellular Ca^{2+} mobilization, while the latter is positively regulated by protein kinase C and results in a further Ca^{2+} increase to sustain phosphoinositide turnover. The presence of the Ca^{2+} ionophores, A23187 and ionomycin also potentiates the phosphoinositide response to ET and ATP. Conversely, KCl in the range of 15 to 55 mM markedly inhibits the phosphoinositide breakdown and intracellular Ca^{2+} increase elicited by these two agonists. These results demonstrate that the phosphoinositide response mediated by these two types of receptors are tightly controlled by intracellular Ca^{2+} levels. Based on the selectivity for adenine nucleotides, it can be concluded that P_{2y} purinoceptors are expressed in C_6 glioma. Purinoceptors in glioma cells, like ET receptors, are coupled to both phospholipase C and Ca^{2+} influx and show homologous desensitization via a protein kinase C-independent mechanism. Electrophysiological studies of the Ca^{2+} channels operated by ET receptors and purinoceptors in C_6 glioma will be conducted by Dr. Hough in collaboration with Dr. M. Rogawski. In another study using cultured cerebellar astrocytes, Dr. Lin has demonstrated the existence of bradykinin receptors which are coupled to phospholipase C. When these astrocytes are subjected to protracted treatment with phorbol esters to deplete protein kinase C, the bradykinin-induced phosphoinositide turnover is greatly potentiated. These results suggest that protein kinase C plays a prominent role in the negative-feedback regulation of the bradykinin-evoked phosphoinositide effect.

In collaboration with Dr. Carmine Coscia's group (St. Louis University), Drs. Chuang and Gao have explored the molecular details of the up-regulation of δ-opioid receptors in hybrid neuroblastoma NG108-15 cells treated with the opioid antagonist naltrexone for 2 days. In this paradigm, the up-regulation is associated with an increase in ^{3}H-DADLE and ^{3}H-diprenorphine B_{max} values in both light and heavy membrane fractions derived from sorbitol gradient centrifugation of the cell homogenate. In contrast, a 5-minute exposure to the opioid antagonist, naltrexone or ICI 174864 induces a transient down-regulation of δ-opioid receptors prior to their up-regulation. This down-regulation is accompanied by a loss in the heavy membrane population of receptors and an increase in binding sites in the light membrane fraction, indicating that internalization of receptors has occurred. Naltrexone and the δ-specific antagonists ICI 174864 and naltrindole also diminish the specific activities of the lysomal enzymes, β-glucuronidase and β-hexoseaminidase. Pretreatment of cells with concanavalin A blocks both the down-regulation and the alterations of lysosomal enzyme activities. These data suggest that the initial process of up-regulation of the δ-opioid receptors by

antagonists entails down-regulation that may involve changes in lysosomal enzyme activity.

In conjunction with Dr. Coscia's group, the team has identified novel intracellular δ-opioid binding sites associated with the nuclei of NG 108-15 cells. These nuclear δ-opioid binding sites have been localized by immunohistochemistry on cryostat sections of these cells with an anti-opioid receptor antibody and the binding of ^{3}H-DSLET, ^{3}H-DADLE and ^{3}H-diprenorphine to the highly purified nuclear preparations. Opioid binding sites have also been shown in nuclear sub-fractions from NG108-15 cell cultures. Opioid agonists ^{3}H-DADLE and ^{3}H-DSLET bind with high affinity to nuclear membranes and with lower affinity to chromatin. In contrast, the high affinity binding sites for the partial agonist ^{3}H-diprenorphine are predominant in chromatin, while low affinity sites are in the nuclear membrane. Accordingly, GppNHp sensitivity of ^{3}H-DADLE binding is detected in nuclear membranes but not in chromatin. Both agonist and partial agonist binding sites in the nuclear membrane and chromatin are abolished by the treatment of cells with cycloheximide. Taken together, the results suggest that NG 108-15 cells contain newly synthesized G protein-coupled δ-opioid receptors in nuclear membrane and internalized, uncoupled opioid binding sites in chromatin. These nuclear δ-opioid receptors may participate in opioid functions that require gene transcription such as opioid tolerance and dependence.

Dr. Stephen Gucker (a NRC Fellow) has used a related neuroblastoma hybrid cell line, NCB-20 (which shares the same neuroblastoma parent with NG 108-15) to investigate the role of G protein in the desensitization and down-regulation of mAChRs. NCB-20 cells possess both m_1- and m_4-mAchRs which are presumably coupled to the stimulation of phospholipase C and the inhibition of adenylate cyclase, respectively. In pilot experiments, Dr. Gucker has demonstrated that upon long-term exposure, some mAChR agonists, such as carbachol, induce mAChR down-regulation, but other agonists, such as pilocarpine, fail to induce the same effect. Digitonin-permeabilized cells and stable GDP and GTP analogues will be used to determine whether this distinction between different agonists is due to differences in their ability to activate G proteins. The second messenger efficacy of different agonists will also be examined for its possible role, if any, in mAChR down-regulation. In addition to direct receptor binding, mRNA levels of mAChRs will be examined using different agonists and under different conditions of G protein activation. Finally, the identity of G proteins potentially involved in receptor down-regulation will be examined by using antisense oligonucleotides directed against specific G protein alpha subunits in order to block or reduce their expression in NCB-20 cells.

In conclusion, during this fiscal year the team has made a number of important findings which include the paradoxical regulation of 5-HT_2 receptor binding sites and their receptor mRNA by 5-HT_2 receptor agonists and antagonists in CGCs, the surprising neuroprotective effect of NMDA against neurotoxicity induced by glutamate and CBZ, the neurotrophic action of lithium, ET and THA effects on mAChR expression, and the presence of novel δ opioid receptor binding sites in the nuclei of neuroblastoma cells. These discoveries have advanced our understanding of possible mechanisms underlying the therapeutic and side effects of psychotropic drugs, and potential mechanisms involved in various mental illnesses and drug tolerance and dependence. These studies may eventually lead to development of new

or improved modalities for the treatment of some mental diseases related to neurotransmitter receptor malfunction.

Summary of 1991-92 Annual Report

Introduction:

A principal goal of our work is to elucidate the molecular and biochemical mechanisms of physical and emotional stress and their relevance to major psychiatric, endocrine, and inflammatory disorders.. We hope to further define the molecular and biochemical bases of diseases that occur as dysregulations of the stress response and to develop more rapid and specific therapeutic interventions based on neuroendocrine pharmacologic modulation of the major effectors of the stress response. A long-term goal is to utilize the information gained regarding pathophysiological features of these disorders to focus on candidate genes whose dysregulation confers susceptibility to this illness. Our work is hypothesis driven, proceeds in parallel on the clinical research unit and in the basic laboratory, and requires the close collaboration and cooperation of neuroendocrinologists, psychiatrists, molecular biologists, neurobiologists, and neuropharmacologists.

Pathogenesis of Melancholic Depression:

Because of the clinical presentation of melancholic depression, this illness was the first in a series that we studied as a potential syndrome that occurred as a consequence of the dysregulation of the generalized stress response. Hence, melancholia is presented as an accentuation and prolongation of the hyperarousal characteristic of the generalized stress response. Subjectively, this hyperarousal presents as an organized state of anxiety about the self, reflected in a pervasive loss of self-esteem and inappropriate guilt. Physiologically, there is a facilitation of arousal-producing neural pathways, evidenced by increased vigilance and focused attention, and by activation of the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system. Melancholia is also associated with a concurrent inhibition of the neural pathways subserving vegetative functions such as reproduction, sexual behavior, and feeding.

Our laboratory has differentiated to study two of the principal stimulatory effectors of the generalized stress response, namely the corticotropin releasing hormone and the locus ceruleus-norepinephrine systems. Studies in experimental animals show that CRH serves not only to activate the pituitary-adrenal axis, but also to coordinate a series of other physiological and behavioral responses adaptive during stressful situations. These include activation of the sympathetic nervous system and pathways subserving vigilance, cautious avoidance, and anxiety, and inhibition of vegetative pathways subserving growth, reproduction, sexual behavior, and feeding. Glucocorticoid secretion during stress serves primarily to counter-regulate the CRH and LC-NE systems to prevent pathological consequences of an unrestrained stress response. Glucocorticoids also participate in the inhibition of the hypothalamic-pituitary-gonadal axis at all levels, in the re-direction of energy, and in the restraint of the immune system.

In a series of studies conducted by Drs. Mitchel Kling, Philip Gold, Julio Licinio, and David Michelson, we advanced many lines of evidence indicating that the hypercortisolism of melancholia reflects hypersecretion of CRH. First, we showed that the ACTH responses to synthetic CRH were attenuated in melancholia and correlated negatively with basal hypercortisolism, indicating intact glucocorticoid negative feedback upon the pituitary. This evidence suggestive of a central locus for hypercortisolism in depression appeared simultaneously with our findings that a continuous infusion of CRH to healthy volunteers could reproduce the 24-hour basal circadian pattern and the magnitude of hypercortisolism typically seen in melancholia, and with our finding that CSF CRH levels in patients with melancholia correlated positively with indices of basal hypercortisolism. Utilizing the technique of contiuous lumbar drainage for 30 hours in patients with melancholia and controls, we also showed that the CRH was hypersecreted into the CSF, with both a phase advance and an increase in pulsatile episodes/24 hours. secretion into the CSF. We next showed that patients with melancholia showed an exaggerated pitutiary-adrenal response to the synthetic glucocorticoid antagonize RU

486. The latter is known to promote the secretion of plasma ACTH and cortisol via disinhibition only during times of active CRH secretion.

The data suggesting hyperfunctioning of hypothalamic CRH neurons in patients with melancholia has stimulated our group to conduct a series of studies exploring the regulation of the hypothalamic CRH neuron. We have examined the effects of a variety of neuropeptides, neurotransmitters, inflammatory mediators, and other informational substances, and our data is summarized as follows: Excitatory Stimuli: Neurotransmitters and Neuropeptides: We have shown that norepinephrine (via the alpha 1 and 2 receptors), neuropeptide Y, (which is co-secreted with norepinephrine), serotonin (via 1A and 5HT 2 receptors), and acetylcholine (via both muscarinic and nicotinic receptors) stimulate CRH secretion. Vasopressin not only acts synergistically with CRH at the pituitary corticotroph cell and in the brain, and is co-secreted with CRH under some circumstances, but also stimulates the release of CRH via V2 receptors. Cytokines and Eicosinoids: We have shown that epidermal growth factor, platelet activating factor, tumor necrosis factor, and several eicosinoids are potent stimuli to the CRH neuron. We have also replicated the work of others with our experimental model showing stimulatory effects of IL-1. The responses to IL-1 and TNF-a could be inhibited by prostaglandin synthesis blockade. None of these substances could stimulate pituitary ACTH secretion in vitro during the incubations at concentrations lower than 10-7m. As Dr. Sternberg will note, these substances could reflect links between the immune-inflammatory response and the CNS, though they may also play an auto/paracrine role in the regulation of the CRH neuron. Inhibitory Stimuli: Neurotransmitters: GABA inhibits the release of CRH via both GABA A and GABA B receptors. Benzodiazepines also restrain the CRH neuron. Alprazolam, which is also a PAF antagonist, is 100 times more potent than diazepam. Feedback regulators: Glucocorticoids inhibit CRH release in a classic negative feedback loop. Arcuate nucleus POMC fragments (including ACTH, b-endorphin, alpha MSH and CLIP), inhibit CRH release via short-loop negative feedback. Using a human-rat CRH antisera that did not cross-react with ovine CRH, we also showed that CRH restrains its own release via an ultra-short negative feedback loop .

A potential role for CRH in the exacerbating course of recurrent affective illness:

Drs. Mitchel Kling and Philip Gold, in collaboration with Dr. Robert Post, have recently advanced a series of studies suggesting that CRH may play a role in the gradual exacerbating course of recurrent affective illness. We have previously shown that the icv administration of CRH to the rat produces limbic seizures that cross-sensitize with electrically-kindled seizures. On the basis of these data and a series of additional studies to be presented by Dr. Kling, we suggested that CRH might play a role in the exacerbating, recurrent course of major affective disorder. Among these findings include Dr. Kling's data that in contrast to controls who show a negative correlation between CSF CRH and age, patients with melancholia show a positive correlation between these parameters. These findings are compatible with our previous data and those of others showing that in contrast to controls, patients with major depression show an increase in mean 24 hour urinary free cortisol excretion with advancing age. To further explore the potential role of CRH in kindling and sensitization phenomena in clinical studies, in collaboration with Dr. Robert Post, we administered procaine to volunteers and patients with affective disorder to monitor pituitary-adrenal and behavioral responses. We noted that procaine produced dose-dependent increases in ACTH and cortisol secretion in both patients and volunteers in association with mood and/or psychosensory changes. To explore the potential mechanism of procaine's effects upon the pituitary-adrenal axis, we assessed the effects of this agent as well as other local anesthetics such as lidocaine and cocaine on in vitro release of CRH from hypothalami and of ACTH from cultured pituicytes. We noted that procaine produced a dose-dependent increase in CRH release but had no effect on the pituitary corticotroph. Interestingly, the effect of procaine was blocked by carbamazepine but not by a number of other agents, including alpha 1 and alpha 2 blockers, serotonin 1B blockade, and nicotinic and muscarinic blockade. We also noted that carbamazepine was capable of inhibiting the responsiveness of the CRH neuron to a variety of stimuli, though it had no intrinsic effect on the

CRH neuron when given alone. Moreover, we showed that carbamazepine had a weak direct stimulatory effect upon the pituitary corticotroph cell. These findings probably account for our in vivo data that carbamazepine administration to non-stressed rats causes a moderate increase in pituitary-adrenal function, but when given to stressed rats, produces a decrease in pituitary-adrenal function. Similarly, carbamazepine administration to normals causes a slight increase in plasma ACTH and cortisol secretion. When given to depressed patients who are hypercortisolemic, carbamazepine decreases plasma ACTH and increases the pituitary-adrenal response to exogenous CRH; conversely, when given to eucortisolemic depressed patients, basal ACTH and cortisol values rise in association with a fall in plasma ACTH and cortisol secretion during the administration of CRH. These data suggest that one possible mechanism of carbamazepine's capacity to exert prophylactic efficacy in affective disorder is to buffer stress responsive systems and promote the establishment of an equilibrium in their functional activity.

To further explore the potential role of CRH on neuronal kindling, we pretreated rats with icv CRH antisera. Our preliminary data show that this intervention was able to attenuate the development of electrically induced kindling. Taken together, these data suggest that repeated activation of the CRH system over the course of recurrent depression could sensitize underlying limbic substrates to influence the natural history of the illness and supports the idea that a principal neuropeptide involved in promoting behavioral and physiological arousal is involved in a process which is known to show cross-sensitization with stressful stimuli. To follow up on these findings, Drs. Mark Smith and Harvey Whitfield have embarked upon a series of studies to explore the effects of electrical kindling on the expression of genes of interest utilizing in situ hybridization. Our data to date show that electrical kindling causes a significant increase in CRH mRNA expression in the amygdala lasting long after the last stimulus is applied. This long-lasting effect on the expression of the CRH gene in the limbic system is of potential interest in the light of our postulates regarding the role of CRH in affective disorder and in the therapeutic actions of carbamazepine.

We have also shown that electrical kindling increases the expression of CRH mRNA in hippocampal interneurons where it is not ordinarily expressed. These are GABAergic interneurons in which CRH is now co-expressed. In the light of data that CRH inhibits the release of GABA from neurons in which the two peptides are co-expressed, we suggest that CRH may play an excitatory role under these conditions by counter-regulating GABAergic neurotransmission in the hippocampus, in keeping with previous data showing that CRH in other brain regions inhibits the release of co-contained GABA.

Concomitant activation of the LC-NE system in melancholia:

The putative activation of the CRH system in major depression also seems associated with a concomitant activation of the LC-NE system. Accordingly, melancholic patients show normal or increased levels of norepinephrine in the cerebrospinal fluid (CSF), and increased levels of CSF and urinary 3-methoxy-4-hydroxy phenol glycol, a principal metabolite of norepinephrine. As a corollary, successful responses to antidepressant medication are consistently associated with decreases in CSF and plasma MHPG, while pre-clinical data indicate that the monoamine oxidase inhibitors and tricyclic antidepressant agents decrease the firing rate of the locus. Our data that CSF CRH correlates positively with CSF NE in volunteers and with a variety of indices of noradrenergic function in patients with depression supports the idea of a linkage between the activation of the CRH and LC-NE systems in controls and patients with melancholia. The idea of a concomitant activation of the sympathetic system in patients with melancholia is also supported by the findings of Dr. Mitchel Kling, who showed an increased rate of norepinephrine spillover into arterial plasma in patients with melancholia.

The idea that melancholia is associated with a concomitant activation of the CRH and LC-NE systems that have escaped their usual glucocorticoid-mediated counter-regulation is supported by the basic studies of Dr. Brady and her colleagues showing a chronic but not acute tricyclic antidepressant-induced suppression of the expression of the CRH gene in the PVN and

of the TH gene in the LC, in association with a significant increase in type 1 GR mRNA in hippocampus.

Differential Pathophysiology and Diagnosis of Melancholia and Cushing's Disease: A Clue to the Pathophysiology of Atypical Depression

One of the persistent problems in clinical neuroendocrinology has been determining the differential diagnosis between major depression and Cushing's disease. Indeed, because depression can present with hypercortisolism of the magnitude of that seen in Cushing's disease, while patients with Cushing's disease can present with major depression, the two entities can be impossible to distinguish from one another. On the basis of the overlapping clinical and biochemical symptomatology, some have suggested that the two entities share pathophysiological features. In his 1973 Sir Henry Dale Lecture, Grant Liddle termed the problem posed by the differential diagnosis and pathophysiology of major depression and Cushing's disease as one of five enduring endocrinologic enigmas. We have conducted a series of studies to explore the differential pathophysiology and diagnosis of these two illnesses. The first of these involved exploring the functional integrity of the pituitary corticotroph cell by administering ovine CRH. In patients with depression, plasma ACTH's were attenuated in proportion to the degree of basal hypercortisolism, indicating that the pituitary corticotroph cell in this disorder was appropriately restrained by glucocorticoid negative feedback. On the other hand, patients with Cushing's disease showed exaggerated ACTH responses to ovine CRH despite basal hypercortisolism, indicating that the pituitary corticotroph cell was grossly unresponsive to the the feedback effects of the glucocorticoids. These findings represented the first clinical data in which responses obtained in patients with depression and Cushing's disease went in the opposite direction, and the limited overlap in the responses rendered the ovine CRH test as an extremely useful one in ascertaining the differential diagnosis of these entities. To date, our data show less than 18% overlap between the two groups.

Our studies in patients with Cushing's disease also revealed that while the pituitary was grossly unrespnsive to glucocorticoid negative feedback, the hypothalamic CRH neuron was suppressed by long-standing hypercortisolism. First, we showed that post-operative patients with Cushing's disease, who frequently show adrenal insufficiency, manifested attenuated, but clear ACTH responses to exogenous CRH, indicating that their adrenal insufficiency reflected a CRH neuron suppressed by long-standing hypercortisolism rather than a pituitary corticotroph cell incapable of responding to endogenous CRH. Third, we showed that the ACTH response to exogenous ovine CRH in post-operative patients with Cushing's disease could be enhanced by priming with the pulsatile administration of synthetic human CRH given experimentally to reproduce the pattern of naturally occurring CRH pulsatile secretion. In this regard, it is a well established fact that pituitary hormonal responses to hypothalamic releasing factors require priming by prior exposure to the pulsatile release of these releasing factors. In additional support of the idea that the hypothalamic CRH neuron in Cushing's disease was suppressed by long-standing hypercortisolism, we showed that the levels of CRH in the CSF of patients with Cushing's disease were profoundly reduced.

These findings of a pathologically supressed hypothalamic CRH neuron in patients with Cushing's disease occurred in association with our data that the depression in patients with this disorder was almost always of the atpical variety, which in contrast to melancholia, is associated with lethargy, fatigue, hypersomnia, and hyperphagia. Moreover, we found that this hypothalamic CRH deficiency can take as long as six or seven months to resolve, so that long-term suppression of the CRH neruons occurs as a consequence of the long-standing hypercortisolism of Cushing's disease. Parenthetically, we noted that patients with Cushing's disease followed longitudinally in the post-operative period showed a high incdence of melancholic derpession during the time when we documented a return of the functioning of the CRH neuon, as assessed by a battery of functional studies of the functional integrity of the hypothalamic CRH neuron.

Hypothethical Pathogenesis of Atypical Depressive Syndromes Across the Boundaries of a Variety of Medical and Psychiatric Syndromes

In contrast to the intense hyperarousal characteristic of melancholic depression, the syndrome of atypical depression seems to represent an excessive counter-regulation of the generalized stress response. The facilitation of pathways subserving arousal and attention in melancholic depression is replaced with apathy, lethargy, and passivity in atypical depression. Similarly, the inhibition of pathways subserving vegetative functions in melancholia contrasts with the hyperphagia and hypersomnia that are among the defining characteristics of atypical depression. This syndrome is not only frequently diagnosed as a primary psychiatric (depressive) illness, but also commonly occurs across the boundaries of a variety of medical illnesses including Cushing's disease, the Chronic Fatigue Syndrome, hypothyroidism, and seasonal affective disorder. Our hypothesis regarding the possible hyporesponsiveness of the effectors of the generalized stress response in this disorder was stimulated by our demonstration of the concomitant activation of the CRH and LC-NE systems in melancholia, and coincided with our demonstration that the atypical depression of Cushing's disease seemed clearly associated with long-standing suppression of the CRH neuron. As we have pursued our studies in these illnesses, our data indicate that, like the anemias, the syndrome of atypical depression in disparate illnesses reflects a common pathway defect deriving from different pathophysiological mechanisms.

We cannot fully account for the fact that the identification of hypocortisolemic/and or CRH deficient states has come so long after the routine identification of hypercortisolism as a concomitant of behavioral disturbances. Methodological issues certainly play a role, because it is far more difficult to document hypocortisolism, whether central or otherwise, than acute or chronic hypercortisolism. Hence, the basal circadian pattern of pituitary-adrenal function is such that it is not abnormal for cortisol levels to be undetectable at certain times of day, while we have shown that plasma ACTH may be normal or even reduced in certain forms of central hypercortisolism. Perhaps a first major step forward was our development of the CRH stimulation test and our application of this paradigm to determine differential pathophysiological mechanisms in melancholic depression and Cushing's disease, and in the various forms of adrenal insufficiency. Prior to that time there were few specific or systematic means of evaluating the functional integrity of the hypothalamic component of the pituitary-adrenal axis or for establishing patterns of pituitary responsiveness to CRH in the various forms of central adrenal insufficiency associated with either post-operative Cushing's disease or as consequence of neoplasm or trauma. Moreover, our development and application of full-dose response curves for ACTH stimulation, our demonstration of the need for CRH priming of the pituitary corticotroph cell, the refinement of plasma ACTH measurements, and a better understanding of the pulsatile secretion of plasma ACTH and cortisol were all critical to these studies. Recently, we have added additional tools, including the measurement of the 30-hour pattern of neurohormone and neuropeptide secretion into the CSF, the assessment of the functional effects of glucocorticoid blockade, and the dose-dependent effects of arginine vasopressin on pituitary-adrenal function at different times of day.

Chronic Fatigue Syndrome:

The chronic fatigue syndrome is defined by the Center for Disease Control (CDC) as an illness consisting of profound debilitating fatigue lasting six months or longer in the absence of any clearly definable systemic illness, and often associated with feverishness, myalgias, arthralgias, and high titers to a variety of viral antigens. In a study of 30 patients meeting CDC criteria for the Chronic Fatigue Syndrome (CFS) followed longitudinally for over one year at the NIH Clinical Center in the laboratory of Dr. Steven Strauss, we showed that the lethargy and fatigue in patients with the CFS seem to occur in the context of a hypofunctioning CRH neuron. Hence, we showed that despite a significant reduction in evening basal total and free cortisol levels and in 24-hour urinary free cortisol excretion, patients with the CFS showed blunted ACTH responses to ovine CRH. We surmise that the blunted ACTH response to CRH in the

absence of hypercortisolism in patients with the CFS reflects a pituitary corticotroph cell insufficiently primed by endogenous CRH, analogous to data to be presented by Dr. Mitchel Kling in post-operative Cushing's disease patients with adrenal insufficiency. We also showed that patients with the CFS showed exaggerated cortisol responses to low doses of ACTH and blunted cortisol responses to high-dose ACTH administration. These data suggest that in the context of a subtle central adrenal insufficiency, adrenocortical ACTH receptors have grown hyperresponsive to ACTH, but that because of an atrophy of the adrenal cortex due to a central CRH deficiency, the adrenocortical response to high doses of ACTH is attenuated. We have previously seen this pattern of response in patients receiving alternate day glucocorticoid treatment and known to have a partial central adrenal insufficiency on this account.

The symptomatology of the CFS could not only be facilitated by a CRH deficiency, but also by hypocortisolism per se. Hence, hypocortisolism is not only classically associated with fatigue, but also with feverishness, myalgias, and arthralgias. Moreover, in the light of the data to be presented by Dr. Sternberg regarding the role of endogenous HPA function on immune functions, hypocortisolism could also be associated with a generalized increase in immunologic function, including increased titers to a variety of viral antigens. Preliminary data from our studies in patients with CFS also show other indices of immune activation.

Seasonal Affective Disorder: Patients with seasonal affective disorder present with depressions characterized by hyperphagia and hypersomnia. In collaboration with Drs. Norman Rosenthal and Thomas Wehr, who have shown normal 24-hour basal circadian corticosteroid secretion in patients with this disorder, we demonstrated a significant attenuation and delay in the ACTH response to ovine CRH. This delayed pattern is analogous, in part, to what we first described in patients with central adrenal insufficiency secondary to trauma or tumor, and, in part, to our data in patients with Cushing's disease studied after transsphenoidal hypophysectomy with post-operative adrenal insufficiency.

Hypothyroidism:

Hypothyroidism, is often associated with symptoms of atypical depression. Dr. Themis Kamilaris of our group demonstrated that patients with hypothyroidism show responses to ovine CRH compatible with central adrenal insufficiency that consist of exaggerated, delayed ACTH responses to CRH, in association with a decreased cortisol response to the ACTH released during the course of the CRH stimulation test.

An animal model for the effects of experimentally-induced hypothyroidism on the functional integrity of the HPA axis in the rat:

In hypothyroid rats, we have utilized a variety of in vivo and in vitro techniques to demonstrate the presence of a central, CRH-mediated adrenal insufficiency. Our in vitro studies show that hypothyroid rats show a decrease in PVN CRH mRNA and content, and a decrease in KCl-induced CRH release form hypothalamic organ culture. We also demonstrated a concomitant decrease in the number of hippocampal glucocorticoid receptors. Studies in pituitary cell culture show a decrease in POMC mRNA and ACTH content, an increase in CRH receptors, and an increased ACTH response of dispersed pituicytes to CRH. Adrenal weights were significantly decreased and there was a subnormal corticosterone response to ACTH by cultured adrenal cells. Corroborating in vivo studies in hypothyroid rats show a significant decrease in CSF and plasma free cortisol concentrations and several findings compatible with central adrenal insufficiency. These include an attenuated response to the central CRH stimulus IL-1, an exaggerated ACTH response to CRH in accordance with an increase in CRH receptors and decreased glucocorticoid negative feedback, and decreased responsiveness of the adrenal cortex to ACTH.

The role of the CRH neuron in susceptibility to illnesses characterized by inflammatory disease and atypical depression:

A bidirectional communication occurs between immune mediators and central nervous system neurohormones that orchestrate the generalized stress response. Peripherally generated cytokines signal several neurohormonal systems in the brain, including hypothalamic corticotropin-releasing hormone (CRH), to participate in maintaining both immunologic and behavioral homeostasis. CRH not only counter-regulates inflammation through pituitary-adrenal activation and the anti-inflammatory action of glucocorticoids, but it also sets in motion a coordinated series of behavioral and physiological responses.

A putative dysregulation in in the hypothalamic-pituitary-adrenal response and this bidirectional communication, by genetic, infectious, toxic, or pharmacological means may confer susceptibility not only to inflammatory diseases, but also to other diseases of the stress response. This concept suggests that disease states characterized by both inflammatory and emotional disturbances may derive from coherent alterations in specific central nervous system pathways. Finally it proposes that neuropharmacological modulation of the CNS components of this bidirectional communication may potentially be applied in the treatment of traditional inflammatory disorders.

We have established the physiologic significance of the immune system - central nervous system feedback loop in our initial studies in Lewis (LEW/N) and Fischer (F344/N) rats, in which we have shown that susceptibility to inflammatory arthritis is related to a defect in the central component of this negative feedback loop, resulting in deficient CRH responses to challenge with a variety of inflammatory and stress mediators. We have now extended these studies to show that the LEW/N CRH hyporesponsiveness, relative to other strains, is profound, and extends across a variety of neurotransmitters and behavioral stressors; it occurs as early in ontogeny as postnatal Day 14, and is associated with a variety of defined behavioral patterns, consistent with the differential HPA axis responsiveness. Thus, the LEW/N rat provides a genetic and developmental model for the analysis of the association between relative CRH hyporesponsiveness susceptibility to inflammatory disease. Our work in progress in this area has focussed on the specific molecular and biochemical mechanisms of LEW/N CRH hyporesponsiveness, particularly regulation through the benzodiazepine/GABA, 5-HT1A and glucocorticoid Type 1 and type 2 receptor systems. We have also focused on further defining the behavioral responses of LEW/N and F344/N rats in order to identify a relatively non-invasive instrument to accurately characterize the behavioral phenotype of LEW/N x F344/N F1 and F2 offspring. This data will be used in genetic studies correlating the behavioral and HPA axis response phenotypes with the phenotype of susceptibility to inflammatory disease in order to determine whether these phenotypes are coordinately controlled by one or more genes.

We also explored whether the relative hypoactivity and hyperactivity of the CRH neuron in LEW/N and F344/N rats were associated with behavioral differences in these histocompatible strains. These studies were undertaken in the light of data that CRH was not only a principal stimulus to the pituitary-adrenal axis, but also was capable of setting into motion a concerted series of other physiological as well as behavioral changes which have been construed as adaptive during stressful situations. Hence, the central administration of CRH to the rat activates the sympathetic nervous system, faciliates pathways that subserve arousal and cautious avoidance, and restrains pathways subserving the program for growth and reproduction. In the course of these studies, we have found behavioral differences as well as differences in neuroendocrine responses to behavioral stressors in LEW/N and F344/N rats, consistent with their relative hypo- and hyper-CRH responsiveness: LEW/N rats exposed to a variety of behavioral stresses, including swim stress, restraint or ether stress exhibit profoundly blunted plasma ACTH and corticosterone responses to these stresses compared to F344/N rats. In open field studies LEW/N rats show greater exploratory behavior, consistent with a diminished CRH behavioral effect. Preliminary behavioral assessment of LEW/N and F344/N rats after intracerebroventricular (i.c.v.) administration of CRH indicate that LEW/N rats are more sensitive to the behavioral effects of low dose CRH than were F344/N rats. The latter finding is

consistent with chronic under-secretion of CRH in LEW/N rats. Preliminary studies also indicate that these strains also differ dramatically in response to acoustic and tactile startle.

These studies thus show that these two strains differ significantly in several behavioral paradigms which reflect in part activity of the HPA axis. They suggest that the CRH neuron as a central nervous system element responding to peripherally-generated inflammatory mediators may play a dual role in the rat during the stress of injury or inflammation. The first of these is an immunologic role, in recruiting the pituitary-adrenal axis counter-regulation of the immune response, with the effect of preventing it from over-shooting; the second is a behavioral role, in promoting the kind of cautious avoidance that can be construed as adaptive in the context of acute injury or inflammatory process.

The L-Trytpophan- Eosinophilia Myalgia Syndrome: Potential role for toxic interruption in the immune system CNS-counterregulatory loop

While the LEW/N - F344/N rat model provides a tool for understanding some of the mechanisms and consequences of genetic perturbations of the immune system - CNS counter-regulatory loop, the L-tryptophan eosinophilia myalgia syndrome (L-TRP EMS) provides a tool for defining the mechanisms and consequences of chemical or toxicological interruptions of this loop. In this regard, L-TRP EMS represents a prototypic inflammatory/autoimmune disease initiated by exposure to a neurotransmitter-related compound. Thus, the second major focus of this lab has been directed at analysis of the pathogenesis of the L-tryptophan eosinophilia myalgia syndrome at a clinical, toxicological and molecular level. These studies also have important public health implications not only in terms of understanding the causes of this epidemic, related to ingestion of impure L-TRP, but also in preventing such future occurrences and in development of specific treatment of the current syndrome. In addition, our data suggesting that the L-TRP EMS could be triggered by the activation of an endogenous receptor (vide infra) may provide information regarding the pathogenesis of a variety of fibrosing illnesses and provide new means for their treatment.

Our specific studies in in the pathogenesis of L-TRP EMS include clinical biochemical studies of L-TRP metabolism in patients who had developed the syndrome compared to controls; development of the first animal model of the syndrome in LEW/N rats; *in vitro* studies of pharmacologic effects and receptor binding characteristics of the synthetic contaminants; effects of the impure L-TRP and synthetic contaminants on CRH mRNA and other aspects of HPA axis function. These studies stemmed from Dr. Sternberg's initial studies of L-TRP metabolism (N.Engl.J. Med., 1980) in patients who had developed the identical syndrome in relation to ingestion of L-5-hydroxytryptophan (L-5-HTP). Our understanding of the pathogenesis of the syndrome has evolved from my initial hypothesis in 1980 that the biochemical abnormality associated with the syndrome (elevated plasma kynurenine) is related to an inborn error of L-TRP metabolism. Our current understanding indicates that the elevated plasma kynurenine, seen also in the L-TRP EMS patients of 1989, is secondary to exposure to an inflammatory stimulus; that inflammation in this syndrome is initiated by exposure to one or more of the L-TRP-related impurities in the implicated L-TRP; that HPA axis suppression associated with exposure to this impurity may play a role in amplification of expression of inflammation; and that the impurity may act through a specific receptor-mediated mechanism.

In November 1989, an epidemic of severe myalgia and eosinophilia was reported to the CDC and the New Mexico State Health Department. The syndrome occurred in patients who had taken L-tryptophan (L-TRP) which was readily available over-the-counter, and prescribed for problems including sleep and depression. Ultimately over 1500 patients in the United States were found to be affected, and to date, 31 deaths have been attributed to the disease. Patients were reported in all 50 states, but predominated in Western states, including California, Washington, Oregon and New Mexico and also clustered in New York, New Hampshire, Minnesota and South Carolina. The distribution of cases is thought to reflect the usage pattern of L-TRP containing products. The epidemic peaked in November 1989, and dramatically fell at the time of the FDA

recall of L-TRP, in November 1989. The epidemiologic data strongly indicated that the syndrome was linked to ingestion of L-TRP manufactured by a single Japanese company, Showa Denko, K.K. (SD). The majority of persons who became ill took L-TRP which had been manufactured during a limited period of time preceding the epidemic in the fall of 1988 to summer of 1989. During this period, SD had simultaneously changed several steps in the manufacturing process of L-TRP, including introduction of a new strain of bacteria, and alteration in the filtration procedure used to purify the compound.

Chemical analyses indicated that the implicated L-TRP contained more than 30 compounds in addition to L-TRP, including compounds structurally related to L-TRP (indoles and beta carbolines), and compounds related to antibiotics (bacitracin). Although several of these compounds in the L-TRP preparation were associated with the development of EMS, one of these compounds, called Peak 97, or Peak E, was most highly statistically associated. Peak 97 was identified as a molecule composed of two tryptophans linked together, now called 1,1'-ethylidenebis[tryptophan] or EBT. In the acid conditions of the stomach, at ph 2, EBT decomposes to a racemic mixture of 1S, 3S tetrahydro b carboline 3 carboxylic acid (SbC) and 1R, 3S tetrahydro b carboline 3 carboxylic acid (RbC).

Clinical Studies: L-Tryptophan Metabolism in L-TRP EMS patients:

Our original description of an illness related to ingestion of L-5-hydroxytryptophan, and the biochemical analysis of tryptophan metabolism described in this paper published in The New England Journal of Medicine in 1980, has now been recognized as the first description of the L-TRP EMS syndrome which re-appeared in 1989 in relation to ingestion of L-tryptophan. In the initial 1980 5-HTP study, plasma kynurenine was elevated in the patient studied compared to controls. This led us to conclude at that time that these patients might have had an inborn error of tryptophan metabolism, pre-disposing them to development of the scleroderma-like illness.

In our initial study of tryptophan metabolism in L-TRP EMS patients in 1989 and 1990, performed in collaboration with Dr. Melvyn Heyes (NIMH) and Dr. Richard Silver (Medical University of South Carolina, see attached New Engl. J. Med. article, 1990), we found once again that compared to healthy controls, plasma kynurenine was elevated in the patients with L-TRP EMS. In addition, however, we determined that quinolinic acid was also elevated in these patients. This pattern of tryptophan metabolism indicated that the alteration in L-TRP metabolism was caused by inflammation, or by exposure to a material which caused inflammation (an inflammatory stimulus), rather than to an inborn error of metabolism. In this regard, if it were an inborn error of metabolism that had caused the increase in kynurenine levels , the most likely enzymatic defect would occur in the catabolic enzymes distal to kynurenine and proximal quinolinic acid in the metabolic pathway. Thus, such a defect would result in elevated kynurenine and normal or low quinolinic acid. A parallel increase in both kynurenine and quinolinic acid, on the other hand, indicates that the increased kynurenine is secondary to induction of the rate limiting enzyme in the pathway, 2,3 indoleamine dioxygenase, with resultant increase in metabolites distal to it. Since it is now known that inflammatory stimuli and mediators such as Interferon gamma induce the rate-limiting enzyme in this pathway, the presence of concurrent increased kynurenine and quinolinic acid suggest the presence of interferon gamma, or exposure to a pro-inflammatory stimulus. These studies could not identify the source of the material which caused the inflammation (i.e. a pro-inflammatory component of contaminated L-TRP versus an environmental trigger), nor could they rule out the possibility that tryptophan itself or its breakdown products might also have contributed to or amplified some of the symptoms of the disease.

We also noted in this study that six of the nine patients with the syndrome either had intrinsic HPA axis hypoactivity (Addison's disease), or were taking drugs (benzodiazepines) which prior data from our group has shown suppresses the HPA axis. In light of our findings in LEW/N rats, this suggested a possible mechanism for the potency of the inflammatory response in this illness, that is, concurrent suppression of the HPA axis due to concurrent HPA axis suppressing medications, or intrinsic hyporesposiveness. Subsequent preliminary studies by the

New York State Health Department, carried out as a result of this suggestion, do suggest that a possible risk factor in development of chronic disease in this syndrome, is concurrent use of psychotropic agents that our group has shown chronically suppress CRH neuronal responses. Furthermore, our ability to reproduce the cardinal features of this syndrome in LEW/N rats (see below), also suggests that a premorbid susceptibility to inflammatory disease related to a suppressed HPA axis, may further contribute to the severity and intensity of the inflammation induced by the impure L-tryptophan.

Development of an animal model of the EMS:

Our model of a central nervous system (CNS) role in the LEW/N rat's susceptibility to inflammatory disease proved relevant to our work with L-TRP-EMS and led to the first and only animal model of this illness. Hence, we recently published in the Journal of Clinical Investigation, that in the LEW/N rat, implicated (case-associated) L-TRP, but not pure USP grade, non-case associated L-TRP or vehicle control, was associated with development of many of the specific pathologic changes in muscle and fascia characteristic of L-TRP-EMS, in the absence of development of eosinophilia (20). We also found that ingestion of implicated L-tryptophan was associated with suppression of corticotropin releasing hormone gene expression in the paraventricular nucleus of the hypothalamus, in association with a fall in plasma corticosterone levels. Hence, these data suggested that intrinsic suppression of the hypothalamic-pituitary-adrenal axis in the LEW/N rat, coupled with a contaminant induced suppression of the corticotropin releasing hormone neuron, might have led to the expression of an eosinophilia myalgia-like syndrome in this animal model. These data not only reflect the first clear-cut evidence that contaminated L-TRP triggers the syndrome, but also provides the means to identify the role and mechanism of specific purified and synthetic contaminants in triggering the syndrome. It also provides the means to identify host factors pre-disposing certain individuals to develop the syndrome, to identify the role of the hypothalamic-pituitary-adrenal axis in susceptibility to the syndrome, to define the role of eosinophils in amplifying the syndrome, and to evaluate new approaches to treatment of the syndrome. To further place this accomplishment in perspective, it should be noted that the toxic oil syndrome, which occurred in Spain in 1980, and affected 20,000 patients, produced a syndrome almost identical to L-TRP-EMS and resulted in thousands of deaths, and that efforts for over a decade to develop an animal model for this disorder failed. It was application of our concepts regarding the susceptibility of the LEW/N rat to the development of inflammatory diusease that was essential to this accomoplishment.

This work takes full circle our initial observation and description of the syndrome in the New England Journal of Medicine in 1980, which is recognized by the scientific community to be the first description of this syndrome in the scientific literature. The current series of studies define the full clinical spectrum of the EMS syndrome; define L-TRP biochemistry in the syndrome and show that the difference in L-TRP metabolism in EMS patients is secondary to inflammation or to an inflammatory stimulus rather than to an inborn error of metabolism; it suggests that an important host factor in susceptibility to the syndrome is a suppressed HPA axis; it establishes the role of contaminant (s) as the etiological trigger in the syndrome; it establishes an animal model in which to further delineate the complex cellular and biochemical mechanisms active in the pathogenesis of the syndrome; it establishes an animal model which further supports the role of the importance of HPA axis suppression in development of the syndrome; it provides an animal model in which specific contaminants can be tested and chemical structures capable of inducing this and similar syndromes can be defined; it provides an animal model which can be used for testing new approaches for the therapy of the syndrome.

Stress-Responsive Neurotransmitters in the Pathogenesis of the Eating and Obsessional Disorders:

In addition to our studies of melancholic and atypical depression, we have also studied the potential role of stress-responsive arousal-producing neurotransmitters in patients with the eating and obsessional disorders. Both groups show an increased family history of major depression and depression is a frequent concomitant of these illnesses. Moreover, some patients with these disorders respond to antidepressant medications.

Anorexia nervosa is the most severe of the eating disorders and is characterized by a striking increase in physical activity and a marked diminution in food intake in the obsessive pursuit of thinness. This obsessive pursuit of thinness often dominates the patient's life to an extraordinary degree, so that life becomes literally organized around the rituals required to maintain a cachectic state. The mortality from this disorder either from the complications of cachexia or suicide is the highest of any psychiatric disorder. Our interest in this illness first arose from the observation that patients with anorexia nervosa invariably show hypothlamic-pituitary dysfunction. In the course of attempting to conceptualize the biological and psychological factors that interact to promote susceptibility to the development, maintenance, and treatment resistance of anorexia nervosa, we helped to clarify the mechanisms underlying the hypercortisolism, central dysregulation of water metabolism, and the hypersecretion of growth hormone in this disorder. Of interest in this regard was our finding that patients with anorexia nervosa, like those with melancholia, show significant hypersecretion of CRH. This finding was publishing in the New England Journal of Medicine in a back-to-back article detailing our findings in melancholia. At that time we suggested that CRH might be a common factor in the pathophysiology and symptom complexes of booth illnesses.

Bulimia nervosa can be an equally debilitating disorder characterized by an uncontrollable pattern of recurrent binge eating and purging. Most patients with bulimia nervosa have strong appetites, cravings for food, and difficulty controlling eating after a meal. Like anorexia nervosa patients, bulimic patients are also pre-occupied with an obsessive pursuit of thinness, and their incapacity to refrain from binge-eating behavior has caused some to refer to them as failed anorexics. During periods of abstinence from bingeing and purging, patients with bulimia nervosa often complain of profound lethargy and fatigue. Bulimia nervosa is a common illness, and some have estimated that 25% of the college-age female population manifest some form of the disorder. Both patients with anorexia nervosa and bulimia nervosa have a greater than expected family history for major depression.

We have developed a model suggesting that a confluence of the following four factors contribute to the susceptibility and natural history of these illnesses: (1) clinical and biochemical manifestations of obsessionalism; (2) a reduction in metabolic rate that interacts with obsessional features to produce a perseverative preoccupation with food intake and body image; (3) primary and/or secondary alterations in the neural mechanisms subserving hunger and satiety that reinforce pathological eating behaviors and; (4) an underlying depression that heightens the eating disordered patient's need to enhance low self-esteem by achieving an idealized body weight. In addition, we postulate that the abstinent bulimic experiences an unpleasant state of hypoarousal not dissimilar from that seen in the various forms of atypical depression that is ameliorated by the bingeing and purging process.

Our data indicate that patients with anorexia nervosa and bulimia nervosa show obsessional symptoms in the range of severity seen in patients with classic obsessive compulsive disorder. These include behaviors such as ritual cleaning, trichotillomania, and repetitive checking. Moreover, their intense preoccupation with food intake and body image can be construed as an obsessional symptom. We have previously reported (*N. Eng. J. Med.*, 308:1117-1123, 1983) that patients with anorexia nervosa show a profound disruption in the osmoregulation of plasma arginine vasopressin associated with hypersecretion of this peptide into the cerebrospinal fluid. In the light of the fact that centrally-directed vasopressin in experimental animals delays the extinction of behaviors acquired during aversive conditioning, we postulated that the hypersecretion of vasopressin into the CNS could contribute to the

obsessive preoccupation that patients with anorexia nervosa have with the potential adverse consequences of eating and weight gain. In the past year, we have shown that normal weight patients with bulimia nervosa and patients with classic obsessive compulsive disorder show qualitatively similar abnormalities in the osmoregulation of plasma arginine vasopressin and in the secretion of this peptide into the CSF. We also identified additional defects in the regulation of CRH and somatostatin secretion in patients with classic obsessive disorder that may be of further relevance to the symptom complex of this disorder.

Our additional clinical and basic studies with arginine vasopressin have continued to support a potential role for this neurohormone in the pathogenesis of the obsessional disorsers. As an example, we showed that among all antidepressants, only fluoxetine caused a sigificant decrease in the secretion of AVP into human CSF. We also showed that fluoxetine was uniquely effective in reducing AVP secretion from hypothalamic organ culture and in reducing AVP mRNA and content in the hypothalamus.

Our data show that normal weight patients with both anorexia nervosa and bulimia nervosa show a reduction in metabolic rate that predisposes them to weight gain, and hence, to potentially pathologic mechanisms for maintaining thinness. In patients with bulimia nervosa studied during a phase of abstinence from bingeing and vomiting, this reduced metabolic rate is associated with a decrement in sympathetic function, augmented parasympathetic tone, and decreased thyroid function. During the phase of active bingeing and vomiting, there is a reversal of each of these defects that may reinforce this pathologic behavior. We postulate that a binge-purge reversible diathesis to weight gain superimposed upon clinical and biochemical manifestations of obsessionalism constitute a particularly toxic confluence that impels patients with the eating disorders into a style of life dominated by pathological eating behavior.

We have recently completed a series of studies exploring neural mechanisms underlying hunger and satiety in patients with the eating disorders. Our data show that patients with both bulimia nervosa and anorexia nervosa show defects in hunger and satiety that either confer susceptibility to their illnesses or complicate recovery. As an example, Dr. Thomas Geriocioti had discovered that patients with bulimia nervosa showed deficient CCK secretion and subjective satiety during the consumption of a normal sized meal that correlated strongly with a deficient sense of satiety after eating. However, during a binge-sized meal, we showed that CCK secretion and subjective satiety were normal. These data suggest that the pathological eating behavior in patients with bulimia nervosa, perhaps as a partial consequence of metabolic and obsessional factors, promoted secondary disturbances in appetite regulation that reinforce abnormal patterns of food intake.

One additional approach we have taken to the study of patients with eating disorders has been to compare and contrast pathophysiological mechanisms in these subjects and those with major affective disorders. As an example, in back-to-back original articles in *The New England Journal of Medicine*, we showed that the hypercortisolism in patients with anorexia nervosa (*N. Eng. J. Med,* 314:1335-1342, 1986) and melancholic depression (*N. Eng. J. Med.*, 314:314:1326-1135) had a similar etiology in the hypersecretion of CRH, and that this defect could contribute to many of their common clinical and biochemical manifestations. On the other hand, the depressive symptomatology in abstinent patients with bulimia nervosa that more closely resembles the syndrome of atypical depression may be alleviated by the repetitive bingeing and vomiting that raises sympathetic tone, augments thyroid function, and activates the hypothalamic-pituitary-adrenal axis.

CLINICAL PSYCHOBIOLOGY BRANCH

Thomas A. Wehr, M.D., Chief

The research activity of the Clinical Psychobiology Branch continues to focus on the identification of causes and new treatments of depressive and manic depressive illness. In this work, we have sought to understand factors responsible for the intrinsic cyclicity of these illnesses and the possible role of biological rhythms in their pathophysiology and pathogenesis. We are currently investigating three populations: patients with seasonal affective disorder, patients with rapid cycling affective disorder, and normal volunteers living in experimentally controlled light-dark cycles.

Seasonal affective disorder is a cyclic form of the illness in which depressive episodes regularly recur during a particular season of the year. Our group pioneered investigations of a type of seasonal affective disorder that is characterized by recurrent winter depression. We showed that winter depression can be treated by exposing patients to bright artificial light, and from this fact we infer that the seasonal cycles of the illness are driven by seasonal changes in the amount of natural light in the environment.

Rapid cycling affective disorder is a cyclic form of the illness in which depressive and manic episodes recur every few days or weeks. In our previous work, we showed that antidepressant drugs that had been prescribed to treat depression were the cause of rapid cycling in nearly half of the patients referred to our program.

In recent basic experiments, we discovered that normal human beings possess archaic physiological mechanisms, similar to those in animals, that enable them to detect and respond to seasonal changes in the length of the day, or photoperiod (in animals these mechanisms are responsible for dramatic seasonal changes in behavior, such as hibernation, migration and breeding). Since these photoperiod-tracking mechanisms regulate the duration of sleep, we are currently investigating whether such mechanisms are responsible for the dramatic changes in sleep duration that occur during the course of seasonal and rapid mood cycles (sleep increases in the depressive phases and decreases in the manic phases of the mood cycles). Identification and evaluation of mechanisms that control sleep duration could be important because manipulations of sleep duration can dramatically alter the clinical state of affective patients, as is discussed below.

Effects of sleep and sleep-deprivation on depression and mania.

About 60% of depressed patients experience temporary remissions if they are deprived of sleep for one night. Many relapse into depression again if they return to sleep, sometimes after only a few minutes of sleep. Patients with manic-depressive illness may become manic after one night's sleep deprivation. Thus, for the majority of depressed patients sleep is depressant and sleep deprivation is antidepressant. The treatment implications of these findings are obvious: 1) sleep deprivation might be used to treat depression, and 2) sleep disruption could be a preventable cause of mania. The research program of the branch has concentrated on two questions about the antidepressant effects of sleep deprivation.

First, can sleep deprivation be used as a practical treatment for depression in the range of patients who seek treatment in a typical clinical setting? To answer this question, Dr. Ellen Liebenluft established a sleep deprivation clinic to which Washington area practitioners could refer depressed patients for sleep deprivation therapy. These patients' responses to the treatment were evaluated with rigorous assessment methods. Sleep deprivation was administered as partial sleep deprivation over a course of four separate nights in a two week period. The study compared the effects of partial sleep deprivation in the first half of the night with partial sleep deprivation in the second half of the night. In all cases, sleep deprivation was used as an adjunct to standard drug therapy. It was used

either to accelerate the response to an antidepressant which had just been prescribed, or it was used to convert drug-non-responders to responders. The results indicate that potentiation of antidepressant drug response with partial sleep deprivation may be useful in the treatment of depression; however, the difference that was expected in efficacies of the two types of sleep deprivation did not emerge from the study.

Second, what is the biological mechanism of the depressant effect of sleep and the antidepressant and mania-inducing effects of sleep deprivation? Answers to these questions could be expected to increase our understanding of the biological causes of mania and depression, and they might lead to fundamentally new types of drug and other treatments—treatments that might be distinguished by a rapid onset of action, as occurs with sleep deprivation, in contrast to existing drugs. We are currently exploring the possibility that mechanisms that control body temperature play an important role in patients' responses to sleep and sleep deprivation. We took up this line of research when we realized that many of the body's responses to sleep (changes in hormones and autonomic functions) are essentially the same as the body's responses to heat. This may be because sleep onset and heat exposure generate the same error signal in hypothalamic temperature control mechanisms. Heat raises hypothalamic temperature relative to the controller's set point, generating a positive ("too warm") error signal. Sleep onset lowers the controller's set point relative to hypothalamic temperature, also generating a positive ("too warm") error signal. With this in mind, we evaluated whether the antidepressant and hormonal effects of sleep deprivation could be augmented by exposure to cold, on the one hand, and blocked or blunted by exposure to heat, on the other. Results with twelve patients studied to date suggest that this hypothesis may be correct.

The hypothesis that heating is depressant, and cooling antidepressant, is consistent with the results of other research in our branch. Dr. Rosenthal showed that phototherapy of winter depression lowers rectal temperature during sleep. Dr. Duncan showed that three classes of antidepressant drugs (a serotonin reuptake inhibitor, a monoamine oxidase inhibitor, and lithium) lower hamsters' brain temperature during sleep (interestingly, neuroleptic drugs, which are antimanic, have an opposite effect). Finally, Dr. Everson showed that chronic sleep deprivation induces robust cold defense responses in rats.

Drs. Rosenthal, Duncan and Everson describe the results of their studies below.

Effects of light and darkness on depression and mania.

About 15% of patients with recurrent depression experience seasonal patterns of recurrence, usually characterized by fall-winter depression. Several years ago we hypothesized that this type of depression was triggered by deficiency of natural light, and we showed that it could be treated successfully by exposing patients to bright artificial light on a daily basis during the winter months. As with sleep deprivation, our research on seasonal depression and phototherapy continues to focus on practical aspects of treatment on the one hand and biological mechanisms on the other.

Phototherapy may be the only psychiatric treatment whose initial site of action (the retina) is known. Therefore, our investigations of mechanisms of light's effects on affective illness have focused on retinal physiology, biochemistry, and pharmacology. Since the neurotransmitters dopamine and melatonin play an important role in the responses of the retina to light, we have investigated whether pharmacological treatment with the dopamine precursor, L-DOPA, could mimic the therapeutic effects of light treatments. Unfortunately, this proved not to be the case. However, in winter depression we found evidence of serotonergic hypersensitivity that was normalized by light treatment, and we replicated this finding. These and other components of our winter depression research projects are discussed by Dr. Rosenthal, below.

Effects of changes in daylength (photoperiod) on normal human biology.

Many animals exhibit striking seasonal changes in their behavior and physiology. In quite a few instances these changes are known to be triggered by changes in daylength, or photoperiod. Several types of animals are able to detect and measure changes in photoperiod by means of biological clocks that track the changing times of dawn and dusk, through the course of the year. According to a classic model, as the timing (phase relationship) of one clock relative to others changes with the changing times of dawn and dusk, changes are produced in the daily patterns of activity, sleep, body temperature and hormones. Seasonal changes in their internal phase relationship can also trigger new behaviors, such as reproductive activity, hibernation and migration, that represent adaptations to conditions that prevail in particular seasons. These new behaviors are often accompanied by and depend on striking changes in the biochemistry, physiology and anatomy of the organism. Although it seems likely that human beings possess similar photoperiodic mechanisms, experiments to evaluate this possibility have not, as far as I know, been conducted. Because such mechanisms are known to have a major impact on important functions in animals, such as reproduction, aggression, metabolism, social activity, hormone secretion and autonomic nervous system activity, their identification in human beings would be fundamentally important.

By placing normal human subjects on light-dark schedules that resemble a natural winter day (10 hours light, 14 hours darkness) we obtained evidence that human biology, like that of animals, is governed by changes in the daily photoperiod. This evidence also indicates that human beings possess the same dawn- and dusk- tracking biological clocks that mediate effects of the photoperiod on animal biology. To date, our experiments indicate that the photoperiod controls daily patterns in human sleep and wakefulness, body temperature, and the secretion of several important hormones. When the duration of night was lengthened, the durations of sleep, nocturnal melatonin (pineal hormone) secretion, nocturnal prolactin (pituitary hormone) secretion, the nocturnal rising phase of cortisol (adrenal hormone) secretion and the nocturnal low temperature phase also lengthened. In addition, sleep separated into two components, an evening bout and a morning bout, with an interval of wakefulness between them. This bimodal pattern, which may seem alien to human beings, is actually ubiquitous in biology, and it is one kind of evidence that has been used to argue that separate biological clocks tracking dusk and dawn, are the fundamental elements of the neural mechanism that enables animals to adjust their physiology and behavior to the changing length of the day and to the changing seasons. From the experiments, it can be inferred that the use of ordinary artificial light, perhaps for 15,000 years or more, has locked human biology into an perpetual summer mode of functioning, and has suppressed and obscured its inherent seasonal rhythms. The impact of this unwitting experiment on human biology and health will be investigated more fully in the future. For example, in a 15-week pilot experiment, we found that exposing human beings to short days and long nights reversibly decreased the size of their pituitary glands by 20%.

Unexpectedly, the photoperiod experiment yielded new information that suggests that the human circadian system is regulated not by just one or two biological clocks, but by multiple oscillators. This conclusion is based on the fact that when the photoperiod was shortened, the timing of various circadian rhythms relative to one another changed. Thus, the timing of the temperature rhythm shifted earlier, relative to both the melatonin and thyrotropin rhythms, and the timing of the thyrotropin rhythm shifted later relative to both the melatonin and temperature rhythms. These observations are consistent with a hypothesis that multiple slave oscillators, possibly under the control of one or two master pacemakers, control different circadian subsystems. In effect, the photoperiod experiment served as a dissecting tool that separated the circadian system into its component parts and revealed its heretofore hidden inner structure.

This basic research in human photoperiodism may help to elucidate the nature and cause of manic-depressive illness. As patients switch back and forth between mania and depression, the duration of their nocturnal sleep phase lengthens and shortens, as occurs in normal volunteers when they are shifted back and forth between long and short photoperiods. Since sleep deprivation experiments have shown that such changes in sleep duration can alter mood and, therefore, may be partly responsible for the symptoms of mania and depression, identification of the mechanism responsible for these changes in sleep duration could be a critical step in understanding the pathogenesis and pathophysiology of the illness. The research on normal human photoperiodism shows that human beings possess a photoperiodic mechanism that regulates sleep duration. The idea that such a mechanism might be responsible for manic-depressive illness seems plausible when one considers that photoperiodic seasonal rhythms in animals induce changes in their behavior and physiology that resemble symptoms and signs of mania and depression. For example, at certain times of year, animals withdraw from the environment, are inactive, sleep more, gain weight and lose interest in sex. At other times of year, they actively explore the environment, sleep less, lose weight, are more aggressive and more interested in sex.

It has never been clear what, precisely, is ill in manic-depressive illness. The discovery of photoperiodism in human beings, and comparative studies in animals, raise the possibility that the illness might reside in an atavistic photoperiodic mechanism that, in a purely natural environment, may have triggered major seasonal adjustments in human beings' energy economy and reproductive physiology. These hypotheses will be explored in the future by exposing affective patients to photoperiod manipulations and monitoring their behavioral and biological effects.

Seasonal Affective Disorder and Light Therapy

Norman E. Rosenthal, M.D.

Since we initially described the syndrome of winter seasonal affective disorder (SAD) approximately ten years ago and showed that it could be effectively treated by exposing those affected by this condition to bright environmental light, we have continued to research both the syndrome and the therapeutic and biological properties of its novel treatment. These themes have been continued in our studies over the past year and are the subjects of the three reports that follow.

In the first report, "Clinical aspects of seasonal affective disorder (SAD)," we describe a follow-up study of patients with SAD, adults, children and adolescents, treated previously in our program. While the clinical characteristics of patients with SAD have been well described, little is known about its longitudinal course. In the second report, "Antidepressant effects of light in winter seasonal affective disorder," we describe our latest multicenter study of the efficacy of a portable, head-mounted light delivery system. This was a follow-up on two earlier studies by our group and others, and was aimed at clarifying a critical question left unresolved by the earlier studies, namely whether the light visor is specifically active or operating by placebo effects only. Our third report, "Neurobiology of seasonal affective disorder and light therapy", deals with our continued attempts to understand the biological abnormalities underlying SAD and the mechanisms by which light therapy exerts its antidepressant effects.

1. Clinical Aspects of Seasonal Affective Disorder (SAD)

In this report, we expand on our previous descriptions of SAD in three separate studies: (1) follow-up study of adult patients with SAD; (2) follow-up study of child and adolescent patients with SAD; and 3) study of changes in psychosocial stressors, cognitive appraisal and coping in winter and summer in adult patients with SAD.

1. The adult follow-up study:

To date, data are available on only 23 of the 68 selected patients, studied in the NIMH Seasonality Studies Program before April, 1985. Sixteen out of 23 (70%) had remained "exclusively seasonal," in that their depressive episodes had continued to be confined to the winter months or they had only used treatment at that time since being discharged from the program. February was the peak month for major depressive episodes. Thirteen out of 23 (57%) continued to use light treatment regularly and successfully each winter after an average of eight years since the diagnosis of SAD had been made. Bipolar II patients tended to require antidepressant medications in addition to lights. All patients continued to experience worsening of their moods during the winter months, and all continued to endorse, and remain invested in, the construct of SAD.

The study thus far shows strong support for the stability of the diagnosis of SAD, and for the continued efficacy of light treatment for most patients with SAD. These observations have implications for the validity of the syndrome, which has been incorporated into DSM-III-R as "seasonal pattern", a term that might be used to modify all forms of recurrent mood disorders. They may be value for clinicians and patients interested in learning the degree to which light therapy can be relied upon and remain clinically effective over time. The fact that a large minority of subjects stopped using light therapy on an ongoing basis may imply that it is not universally helpful in the long run or may reflect the inconvenience of ongoing treatment, as it is currently administered (See report # ZO1 MH 02402 01 CP).

2. Child and adolescent follow-up study:

About seven years ago we described seven children and adolescents with SAD. We have now followed up six of the seven of these patients, all of whom report that they continue to experience some form of seasonal dysfunction, although knowledge about the condition has enabled them to deal effectively with the winter. Five of the subjects have light boxes in their homes and use them in an "as needed" and unstructured manner. All report making conscientious efforts to spend time outdoors during the winter and have developed outdoor hobbies, such as gardening, hiking and water skiing. Two patients report great benefit from fluoxetine, 20-40 mg per day. Patterns of symptomatology for individual cases have remained quite stable over time.

Results obtained so far suggest that SAD, as it appears in childhood and adolescence, is a stable condition that manifests itself symptomatically year after year. This underscores the importance of early and accurate detection in order to prevent seasonal depressions, which impair the child's quality of life and can impede emotional and intellectual development. The finding that most of the children continued to use light therapy in some form and to modify their exposure to natural sunlight by undertaking outdoor activities, suggests the ongoing benefit of these interventions.

Our findings thus far have indicated the importance of learning more about SAD in children and adolescents. There is very little in the literature on this topic and it would be important to document the characteristics and prevalence of seasonal changes in mood and behavior and its response to treatment. We are planning studies to investigate these particular questions in collaboration with researchers in the Child Psychiatry Branch, IRP, NIMH.

3. Stress, cognitive appraisal and coping in SAD:

Although we recognize the influence of the physical environment in the development of depressive symptoms in SAD patients, the influence of psychological factors has not been well studied to date. To address this question, we evaluated 37 SAD patients and 29 normal controls on indices assessing life stressors, cognitive appraisal and coping during both winter and summer. We found that depressive episodes in SAD patients are not generally precipitated by major life stressors. SAD patients showed no

seasonal variation in the number of major life stressors reported and did not differ significantly from normal controls in the number of major life events reported on initial evaluation during the winter. SAD patients did, however, report greater frequency and severity of minor daily stressors than controls during the winter when they were depressed, but not during the summer after they had remitted, though they reported these stressors as more severe than controls even in summer. Examination of cognitive processes involved in coping with stressful events showed no seasonal differences in SAD patients or differences between SAD patients and controls in how they evaluated the significance of stressful events or their perception of available resources and coping options. Finally, like other depressed populations, SAD patients tended to use coping strategies which involved more avoidance, regulation or discharge of affect and less problem solving when depressed. They did not differ from controls, however, when symptoms had remitted.

Results of this study confirm our earlier observations that depressive symptoms in SAD patients are not triggered primarily by psychological stressors or precipitants.

2. Antidepressant effects of light in winter seasonal affective disorder

Since we first showed that bright light could be used as an effective antidepressant in the treatment of SAD, we have performed a number of studies to investigate those formal properties of light necessary for its therapeutic effect, as well as optimal methods of light delivery. Thus, we showed that the antidepressant effect is mediated via the eyes rather than the skin, that it depends upon the intensity of the light source, that an antidepressant response can be observed when light is administered at various times during the day, and that green light is more effective than red light.

More recently, we have addressed the practical problem of the traditional method of administering light therapy by using light boxes, which are safe and effective but cumbersome devices, which are not easily transported. To overcome this inconvenience, we have developed a portable, battery-powered, head-mounted "light visor" in conjunction with researchers at Jefferson Medical College. We have previously tested this device in two separate multicenter studies. In addition to the advantage of portability and convenience, the light from the visor remains in fixed relation to the wearer's eyes during light therapy sessions whereas, when light therapy is administered from a light box, small movements of the head can result in large variations in the amount of light reaching the eyes.

The light visor was licensed and manufactured by the Bio-Brite Company, which provided visors and funding for two earlier studies by means of a Collaborative Research and Development Agreement (CRADA) between the company and the NIMH. The study performed this past year was not done as part of a CRADA with Bio-Brite, though the company provided the visors without charge.

The light visor resembles a baseball cap, from which two incandescent, filtered light sources direct light toward the eyes. In the first visor study, conducted during the winter of 1989-90 at three centers (Bethesda, MD; Nashua, NH; and Seattle, WA) , fifty-five winter SAD patients participated in a parallel design study in which we compared visors of two intensities of white light: 500 lux and 5000 lux. On the basis of earlier light box studies, in which a direct relationship between intensity and efficacy was noted, we hypothesized that the brighter (5000 lux) visor would be more effective than the dimmer (500 lux) one. Patients were treated for either 30 or 60 minutes on arising in the morning. Contrary to our prediction, the brighter visor was not more effective than the dimmer one. Paradoxically, when response rates were compared using stringent response criteria, the dimmer visor was almost superior to the brighter one. Response rates for the two visors were 56% and 27% respectively, the former value comparing favorably with response rates found in some earlier light box studies.

In an attempt to clarify and elaborate on these unexpected findings, we participated in a five-center study, co-ordinated by Dr. Russell Joffe at the Clarke Institute, Toronto. Other centers involved, apart from our own, were University of British Columbia,

Vancouver; University of Utah, Salt Lake City; and McLean Hospital, Harvard University, Belmont, Massachusetts. We tested three different intensities of white light: 3200 lux, 600 lux and 60 lux. Once again, we hypothesized some direct relationship between intensity and efficacy and predicted that the dimmest visor would be the least effective. A total of 105 subjects were studied, 30 of these at the NIMH. Again, we found no difference between the effects of the three visors. Stringently measured response rates of response rates for the 60 lux, 600 lux and 3200 lux visors were 45%, 50% and 54% respectively.

We recognized that there were at least two alternative explanations for the paradoxical findings of the previous years. First, the antidepressant response might have been due entirely to placebo effects. Second, all visors might have exceeded the threshold for a true biological response to light therapy. This past year we undertook a third study in this series in an attempt to dissect apart these two possibilities. Arguing that perhaps the dimmest of the visors in the previous studies were not dim enough, we tested an even dimmer visor (30 lux), emitting red light, which has been shown to be relatively inert biologically, against a brighter (600 lux) white visor. The study was performed at two sites, our own and McLean Hospital, a Harvard University affiliate, and was co-ordinated out of the latter site.

During the winter of 1991-92, 57 winter SAD patients at 2 centers (Bethesda, MD; and McLean Hospital, a Harvard University affiliate) participated in a parallel design study comparing visors of two intensities and different spectra: 30 lux red light and 600 lux white light. Fifty seven subjects were studied at the two centers; 30 of these at the NIMH. There was no difference between the antidepressant effects of the two visors. Stringent response rates for the 30 lux red and 600 lux white visors were 41% and 39% respectively.

We are still unable to say with certainty why visors of widely differing intensities yielded no difference in response rates, both in the present study and in the previous two studies. These findings could be due to placebo effects, true biological effects of light or other non-specific biological effects of the interventions. These different possibilities are discussed in greater detail in the body of the report.

3. Neurobiology of Seasonal Affective Disorder (SAD) and Light Therapy

Although repeated studies have established that bright light is an effective treatment for SAD, the mechanism of its actions remains unknown, as do the fundamental biological abnormalities responsible for the syndrome. We have investigated these questions of mechanism along two separate lines: (1) studies of the eye and its connections to the brain; and (2) studies of brain serotonin systems. The former of these two lines of inquiry is addressed by Dr. Dan Oren in a separate report (# Z01 MH 02611 - 01 CP), while the latter is the subject of the present report.

There is ample reason to believe that brain serotonergic systems are disturbed in SAD. Patients with this condition crave carbohydrates and report feeling activated when they consume sweets and starches. Carbohydrate-rich meals have been shown to increase brain serotonin synthesis in animals, an effect that has been postulated to occur in humans as well. In an earlier study, we fed patients meals rich in either carbohydrate or protein and found, as we had predicted, that carbohydrate-rich meals activated SAD patients but sedated normal controls. In a study, undertaken at M.I.T., SAD patients were found to respond to the serotonin agonist d-fenfluramine to a greater degree than to placebo. In a post-mortem study of human brains, derived from individuals who died at different times of the year, hypothalamic serotonin content was found to drop during the winter months. This could be the physiological basis for certain vulnerable individuals' becoming depressed during the winter.

In the past, we undertook preliminary studies to investigate whether SAD patient would respond differently from normal controls to infusions of the relatively selective serotonin agonist, m-cholophenylpiperazine (m-CPP). We found that SAD patients, when untreated during the winter months, reported being activated to a greater degree following infusions of m-CPP than normal controls. Effective light treatment or the advent of

summer were associated with a normalization of this activation response. In addition, SAD patients showed exaggerated cortisol and prolactin responses to m-CPP, providing further evidence of serotonergic abnormalities in this condition insofar as the secretion of both of these hormones may be regulated in part by serotonin. These hormonal responses were decreased by light treatment in both patients and normal controls.

Although our earlier m-CPP studies of SAD strongly suggested abnormalities in brain serotonergic systems, which are normalized by light therapy, they suffered from certain methodological deficiencies. First. the number of subjects studied was relatively small. Second, we used no placebo infusions. Thus aberrant behavioral and hormonal responses could be interpreted as representing non-specific reactions in a pathological population. Finally, all patients were studied first in the untreated and subsequently in the light-treated condition. An ordering effect might thus have confounded the apparent effects of light therapy. In the present study, we evaluated the effects of m-CPP in 17 SAD patients and 15 healthy controls, in both untreated and light treated conditions in a study that avoided the problems of our earlier investigation. Thus we randomly ordered treatment conditions, used a placebo control and included a larger number of subjects.P

At the time of writing, blood samples are still being assayed for hormone levels, and body temperature profiles are still being analyzed. This report will therefore deal only with the results of behavioral measures. As we had found previously, the "activation-euphoria" subscore increased significantly in SAD patients during the "off lights" condition following infusion of m-CPP, as compared with the "on-lights" condition or with the normal controls under either condition. There were no significant differences between the responses of patients and controls on any other self-rating subscore. Increase in activation following m-CPP infusions in patients in the "off-lights" condition correlated significantly with antidepressant response to light, as measured by the HDRS. In fact, activation following infusion was a better predictor of decrease in the HDRS total score than any individual HDRS symptom at baseline.

The activating and euphoriant effects of m-CPP on untreated patients with SAD is one of the few replicated biological abnormalities described in this condition. This behavioral response appears specific for SAD. Insofar as light treatment reversed this abnormal response, it is possible that light treatment may be acting via the same receptors that are stimulated by m-CPP. Chronic light treatment might desensitize these receptors, thereby diminishing subsequent exposure to the drug. The significant correlation between the activating effects of the drug and the antidepressant response to light, as measured by a standard depression scale, further suggests an association between these two different interventions.

The present study, along with its predecessor and data from other centers, provides strong evidence of the involvement of serotonergic pathways in SAD and its response to light therapy. Further investigations of this possible association are clearly warranted.

Circadian Rhythms, Rapid Cycling Bipolar Disorder, and Gonadal Steroids

Ellen Leibenluft, M.D.

A Controlled Study of the Antidepressant Efficacy of Sleep Deprivation

There is an extensive body of literature to indicate that, in approximately 50% of depressed patients, staying awake for all or part of the night can have an antidepressant effect. While the antidepressant effect of sleep deprivation (SD) has been used widely as a research tool, its clinical application has been limited by the fact that the effect is short-lived, and most patients relapse after a night of recovery sleep. This project, which was completed and terminated this year, was designed to test two possible clinical applications of SD in the treatment of depression: (1) the use of SD to hasten the onset of the

antidepressant action of fluoxetine; and (2) the use of SD to potentiate the action of antidepressant medication in patients who have had a limited response to such medication. The first clinical application could be significant because of the morbidity and mortality that result from the delay between the initiation of antidepressant therapy and the onset of therapeutic action; this delay is commonly a month long and can be as long as three months. The second clinical application is important because approximately 15% of depressed patients are considered treatment-refractory, and have a limited response to conventional antidepressant treatments.

In both of these protocols, as in all SD research, a major methodological problem stems from the fact that patients are aware that they are being sleep-deprived. This complicates the task of designing an adequate control condition for the SD treatment. Since the literature indicates that SD in the second half of the night (late SD, or LSD) is a more effective antidepressant than SD in the first half of the night (early SD, or ESD), these studies were designed to use ESD as a control condition for the more active LSD. Thus, in the first study, we hypothesized that patients randomly assigned to LSD would respond to fluoxetine treatment more quickly than those assigned to ESD, while in the second study we hypothesized that patients randomly assigned to the LSD condition would experience more clinical improvement than those assigned to the ESD condition. In both protocols, patients were sleep deprived for two nights in a row for two weeks and were then followed weekly in clinic for three weeks.

In the first protocol, patients were drug-free at the beginning of the study and were started on fluoxetine 20 mg daily four days before their first night of SD. Twenty-four patients completed the protocol, eleven in the LSD condition and thirteen in the ESD condition. There were no significant differences between the LSD and the ESD group in the course of their response to fluoxetine. Thus, while patients improved significantly over the course of the study, it was impossible to distinguish the relative contributions of fluoxetine, SD, and patients expectations to this clinical change. Therefore, it is unclear whether the SD treatment did in fact hasten the course of the patients' response to fluoxetine.

Patients accepted into the second study had all been on a stable regimen of antidepressant medication for at least eight weeks and were continued on that regimen throughout the study. Twenty-six patients completed this protocol, fourteen in the ESD condition and twelve in the LSD condition. Once again, as in the first study, there was no significant difference in clinical course between the ESD and the LSD groups, so that ESD does not appear to be an adequate control condition for LSD. However, the SD treatment did decrease significantly the patients' Hamilton Depression Rating Scale scores. Therefore, it appears that SD may potentiate the efficacy of antidepressant medications in this relatively refractory group of patients. However, the role of patient expectations cannot be definitively excluded because of the lack of an appropriate control condition. Prolactin, cortisol, TSH and fT3 levels drawn at 8:00 a.m. at baseline and on the mornings after SD varied significantly over the course of the study but were not related to clinical response.

Circadian rhythms in rapid cycling bipolar disorder

Patients with rapid cycling bipolar disorder (RCBD) present both a challenge and an opportunity to psychiatric researchers and clinicians. The challenge stems from the fact that these patients, who experience at least four episodes of depression, mania, or hypomania each year, are often refractory to conventional treatments and suffer significant morbidity. On the other hand, their frequent and dramatic shifts in mood state, accompanied as they are by dramatic changes in the sleep-activity cycle, provide a relatively accessible model for research. Patients with RCBD typically experience hypersomnia when depressed and insomnia when hypomanic or manic. Sleep deprivation studies, including the project described above, demonstrate that sleep deprivation can trigger a switch out of depression

and therefore that changes in the sleep-wake cycle may play a pathogenic role in severe mood disorders. The current project, which is in an early data-gathering phase, is designed to study the sleep-wake cycle and the circadian rhythms of patients with RCBD in order to understand the role that these rhythms may play in the etiology of both RCBD and other forms of affective illness.

Several models could be used to explain the shifting sleep-wake cycles of patients with RCBD. Using a model of a single circadian oscillator, one could hypothesize that the oscillator undergoes successive cycles of phase advance and delay. Data from human free-running experiments would suggest that the duration of a patient's sleep would then depend on the phase position of the temperature rhythm at the time of sleep onset. Alternatively, data from animal studies and from Dr. Wehr's work suggest that sleep duration is regulated by two coupled oscillators, one of which tracks dusk while the other tracks dawn. According to this two-oscillator model, changes in the relative positions of the two oscillators could explain the changing duration of sleep in RCBD.

In this project, we test these alternative hypotheses by studying the circadian rhythms of patients with RCBD. Patients with this illness are admitted to the hospital for brief, intensive chronobiological evaluation twice in the course of their illness: once shortly after switching into a hypomanic state, and once shortly after switching into a depressed state. During a 48-hour stay in the hospital, blood is drawn every 20 minutes through an indwelling intravenous catheter, and patients wear activity monitors and rectal temperature monitors. For the first 20 hours of this evaluation, patients adhere to their normal sleep-wake schedule, and blood is drawn to measure levels of prolactin, growth hormone, T-3, LH, FSH, estrogen, progesterone, testosterone, and sex hormone binding globulin. During the second 28 hours of the inpatient evaluation, patients are kept under constant routine conditions. In these conditions, patients are kept awake in a dim room with limited activity. They are fed meals, along with one-twelfth of their usual daily dose of medication, every two hours. The purpose of the constant routine is to control the confounding (masking) effects of sleep, light, activity, and caloric loading on circadian rhythms. During the constant routine, blood is drawn to measure melatonin, cortisol, and TSH. The unmasked temperature and melatonin data from the constant routine will be used to identify changes in circadian rhythms in the two mood states. In addition, levels of hormones measured in both the naturalistic and constant routine conditions will be used to further characterize the patient's physiology in each of the abnormal mood states.

In most studies, women comprise 80 to 95% of patients with RCBD. The reason for this gender difference is unclear, and there has been little research attention directed at a possible role for gonadal steroids in the pathophysiology of this illness. In this project, as noted above, we measure levels of estrogen, progesterone, LH, FSH, testosterone, and sex-hormone binding globulin in the depressed and hypomanic phases. In addition, premenopausal women with RCBD follow in a systematic way the relationship between their menstrual cycle and mood.

In conjunction with the above project, we are currently designing several other studies which will test the efficacy of several experimental treatments for RCBD while further exploring the etiology of the illness. These studies will use L-thyroxine, leuprolide acetate (a gonadotropin releasing hormone agonist), and bright light as therapeutic agents.

The role of gonadal steroids in regulating circadian rhythms in women

The role of disrupted circadian rhythms in the pathogenesis of affective illness has been a long-standing research interest of the Branch. Two of the affective illnesses that have received much of our research attention----seasonal affective disorder and rapid cycling bipolar disorder---are much more prevalent in women than men. Studies of men and women in temporal isolation indicate that women have shorter sleep-wake cycles than men and spend a greater fraction of their time sleeping. These observations raise the question of whether gender differences exist in the regulation of circadian (and seasonal)

rhythms, and whether gonadal steroids play a role in the etiology of such differences. Indeed, an extensive literature indicates that estrogen and progesterone modulate the behavior of circadian pacemakers in female rats and hamsters, but this question has not been explored in humans.

This project, which is being done in collaboration with the Section on Reproductive Endocrinology of the Biological Psychiatry Branch, studies the circadian rhythms and sleep-activity cycle of normal women in pharmacologically controlled hormonal states. Recruitment for subjects is now beginning. Normal women will be placed on the the gonadotropin releasing hormone agonist leuprolide acetate (Lupron) for three months. This treatment suppresses endogenous gonadotropin and gonadal steroid secretion in a reversible fashion. For the first month on the protocol, women will receive Lupron alone and will therefore be in a hypogonadal state. Subsequently, they will receive estrogen replacement alone for one month, followed by progesterone alone for one month. In each of these conditions, we will monitor the subject's activity level, EEG sleep, and rectal temperature curve. In addition, we will study the circadian rhythms of melatonin, prolactin, and growth hormone in each hormonal condition, as well as performing a constant routine procedure to obtain unmasked temperature data.

The results of this protocol can have theoretical as well as practical implications. While providing information about the role of gonadal steroids in the regulation of circadian rhythms, and possibly in the pathophysiology of mood disorders, the protocol will also generate data on the effects of hormone replacement therapy on the sleep-activity cycle.

An Investigation of Primary Depressives with Secondary Alcoholism

Ellen Leibenluft, M.D.

While affective illness and depressive disorders are known to frequently coexist, the majority of the research in this area has been related to syndromes of secondary depression following periods of alcohol abuse and dependence. In contrast, little research has been done with patients who first experience an affective syndrome and then go on to develop alcohol abuse or dependence. While alcoholism in general is more common in men, this syndrome of primary depression and secondary alcoholism, like depression itself, tends to be more common in women. Clinicians and patients frequently explain this clinical pattern by saying that the patient "self-medicates" her depression with alcohol, but this hypothesis has not been examined systematically. The purpose of these studies, which were completed and terminated this year, was to attempt to characterize patients who have primary depression and develop secondary alcoholism, and to achieve a more thorough understanding of the relationship between their depressive symptoms and their substance abuse.

The first of these studies consisted of a small pilot study in which patients with primary depression and secondary alcoholism were compared to patients with depression alone, and to patients with alcoholism alone. Eleven patients in each group, matched for age, sex, and Global Assessment of Functioning score, received an extensive standardized work-up in which we characterized symptoms, family history, and pattern of drug and alcohol abuse. The results indicate that comorbid patients are significantly more likely than depressed patients to meet DSM-III-R criteria for panic disorder, and that, compared to the alcoholic group, they have significantly higher scores on the Hamilton Anxiety Scale. The comorbid patients also reported a higher percentage of first-degree relatives with a history of drug abuse than the other two groups. Finally, compared to the depressed patients, the comorbid patients had significantly higher hypomania scores, although (with the exception of one comorbid patient, whose elimination from the sample did not change the results) neither the depressed nor the comorbid patients met criteria for bipolar illness. These

results indicate that patients with primary depression and secondary alcoholism may tend to be "trimorbid", with symptoms of depression, anxiety, and alcoholism.

A second study involved the development of a questionnaire to study patients' use of alcohol, carbohydrates, and caffeine in response to depressive symptoms. The questionnaire inquired about patients' use of each of these substances in response to each of the symptoms on the Hamilton Depression Rating Scale, and about the effect that they thought the substance had on each depressive symptom. After establishing test-retest reliability, the questionnaire was administered to normal volunteers (N=26) and to patients in each of four diagnostic categories: seasonal affective disorder (N=117); major depressive disorder (N=35); primary depression with secondary alcohol dependence (N=24); and alcohol dependence (N=16). Patients in the latter two groups did not differ in the reported use and effect of alcohol, so that alcoholics without a history of depression were as likely to report drinking in response to depressive symptoms as were those with a history of primary depression. In terms of caffeine and carbohydrate use, the responses of the patient groups did not differ from each other, but all differed significantly from those of normal volunteers. Discriminant function analysis distinguished alcoholics from non-alcoholics in the relationship between alcoholics' reported drinking, anger, and anhedonia.

Opthalmic Function and Light Sensitivity In Seasonal Affective Disorder and Biological Aspects of Hypernychthemeral Syndrome

Dan A. Oren, M.D

For the past four years we have studied ophthalmic function and light sensitivity in the syndrome of winter seasonal affective disorder (SAD). This year we have continued to explore this avenue of research and we have also undertaken a case study of a patient with hypernychthemeral syndrome—a rare disorder of circadian rhythms last studied in this branch a decade ago.

In a series of clinical experiments (ZO1 MH 02613-01 CP, "Light and the Eye in Winter Seasonal Affective Disorder (SAD)") we have addressed the roles that environmental light and the processing of light by the eye may play in winter depression. The cause and fundamental pathophysiology of SAD is unknown. Although winter depression occurs during the time of year when there is the least sunlight, we did not know if people with SAD develop the syndrome because they are exposed to less light than people without SAD. Therefore, we used a portable, wrist- and lapel-mounted light measurement device to measure ambient light exposure in patients with SAD and normal volunteers. We have also hypothesized that SAD might develop because of abnormal processing of light by the eye and we have carried out experiments to assess ophthalmic function in SAD. Below we summarize the findings from a series of studies of ophthalmic function measuring intraocular pressure, dark adaptation, and electrooculograms in patients with SAD and in normal volunteers.

Our interest in light and the human response to it has led us to study hypernychthemeral syndrome, a rare illness whereby patients with the disorder are unable to respond to the normal cues of the daily cycle of light and dark and instead go to sleep and awaken persistently later each day. To date there have been no descriptions of fundamental biological abnormalities that may account the disorder. In a second set of experiments (Z01 MH 02611-01 CP, "Biological Findings in Hypernychthemeral Syndrome,") we describe beginning efforts to characterize biological abnormalities that may be found in the syndrom.

Light and the Eye in Winter Seasonal Affective Disorder (SAD)

The initial description of SAD noted that the symptoms of this syndrome occur during the months of the year when there is the least ambient light. No comparison has been made, however, between the ambient light exposure of patients who experience SAD and people without SAD. Therefore, we examined light exposure—calibrated to the sensitivity of the human eye—in 13 SAD patients and 13 age- and sex-matched controls using a portable light monitor that was worn on the wrist and measured environmental light levels for a full week. Ambient light exposure did not differ between patients and controls in any of several factors examined. There was a significant correlation, however, between measured length of photoperiod (the illuminated fraction of the day) in patients and their levels of depression. This study suggests that SAD patients are not exposed to less light than people without the syndrome. SAD is therefore less likely to be an "environmental" disease than a consequence of abnormal central nervous system processing of normal environmental light.

We hypothesized that abnormal retinal dopaminergic activity might play an etiological role in SAD. Our prior studies of ocular functioning, using several paradigms of investigation in a large sample of patients did not identify specific abnormalities in SAD patients. This year we continued our investigation of eye pathophysiology by exploring two areas of ophthalmic function that were reported by other groups to be abnormal in SAD. Stojek et al. reported that intraocular pressure was lower in SAD patients than controls and that it could be normalized by light therapy. We examined intraocular pressure in 14 SAD patients and 14 age- and sex-matched normal control subjects before and after light therapy. We found no difference in intraocular pressure between patients and controls or between before and after light therapy. In an attempt to replicate Lam et al.'s report of abnormal low electrooculograms (EOG's) in SAD and to determine if light therapy changes electrooculogram ratios, we measured this variable in 16 SAD patients and 16 matched controls before and after light therapy. Mean EOG ratios were lower in patients than in controls, although there was a great degree of overlap between ratios of patients and controls. We were able to go beyond the data of Lam and colleagues by demonstrating that light therapy had no effect on the EOG ratios. There was no correlation between changes in Hamilton Depression ratings and the EOG ratios.

We and others have hypothesized that altered sensitivity to light may cause predisposed individuals to develop winter depression. Therefore, we evaluated dark adaptation in winter and summer in 11 SAD patients and 19 controls by means of a Goldmann/Weekers Adaptometer. We found no difference between SAD patients and controls, however, and no effect of season on dark adaptation.

Our inability to find any connection between SAD and several parameters of ophthalmic function and only a modest abnormality in EOG adds further weight to our previous work casting doubt on the idea that eye abnormalities contribute to the pathogenesis of SAD. Taken together, this series of studies suggests that the likely loci for the pathophysiology of SAD will be found in brain structures associated with biological rhythms or with depression in general rather than in the eye. The electrooculogram finding remains and awaits elucidation. It may be related to abnormal ophthalmic dopaminergic or serotonergic function, but it also might be an artifact of the examination procedure.

We plan to continue our investigation of electrooculographic abnormalities by repeating these studies in patients and volunteers during the winter and summer seasons. Other eye-related investigations of SAD seem less promising at this time. We do plan to study whether medications that will enhancing light sensitivity or shift the body clock might prove beneficial to patients with SAD.

Biological Findings in Hypernychthemeral Syndrome

Hypernychthemeral syndrome is a rare disorder in which patients find themselves unable to initiate sleep at the same time regularly. Patients with this disorder instead find themselves, on average, going to sleep later and later each day. In this way, their sleep gradually goes around the clock every few weeks. In 1983 this Branch reported the first successful pharmacological treatment of non-24-hour sleep-wake syndrome (hypernychthemeral syndrome). We have conducted a case study analysis of another patient with this syndrome as a window into understanding various processes that control human circadian rhythms. The patient wore a light meter continuously for a week to assess his level and pattern of ambient light exposure. Of possible relevance to his problem, ambient light exposure was less in this patient than in a sample of normal volunteers that we studied for a different project. The patient was later admitted to the research unit for monitoring of daily temperature, hormonal, and sleep architecture rhythms. We observed multiple endocrine and circadian rhythm abnormalities: 1) Expected hour of sleep onset was delayed by two to three hours relative to the normal fall in temperature; 2) TSH levels were abnormal in opposite ways on two occasions. The expected nocturnal rise during one sampling was absent and abnormally high levels were present on another sampling; 3) Testosterone, FSH, and LH levels were all below normal levels; 4) Most remarkable, there was no detectable plasma melatonin, although low urinary levels of the metabolite 6-sulfatoxymelatonin were present. These data provide strong evidence of abnormal pineal gland and hypothalamic-pituitary gland-end organ axis disease, which we plan to study further through endocrine challenge tests. We plan to try various pharmacological approaches to correcting the sleep-wake disorder. This case study-in-progress offers significant potential for furthering our understanding of hormonal regulation of the sleep-wake cycle.

Physiology of Sleep and Sleep Loss

Carol A. Everson, Ph.D.

Sleep is a vital biological process that is implicated in the maintenance of both physical and mental health. To understand its clinical implications for higher-order function, we must first study sleep directly, both to determine how its effects are mediated and how physiological changes are induced by sustained wakefulness.

Prolonged sleep deprivation in the rat causes an unexplained catabolic state, secondary malnutrition symptoms, and mortality. We have determined that the severity of the malnutrition symptoms, as well as survival time, is partially dependent on the nutritional composition of the diet and reflects responses of intermediary metabolism to nutrient and energy availability. Diet composition interacts strongly with sleep deprivation, affecting the time-course and development of pathologies, whereas it exerts a negligible influence on body weight regulation under normal conditions.

The vital function impaired by sleep deprivation that results in death in animals has long been a mystery. Its identification would tell us not only what type of physical harm is preventable with sleep, but it might also point to an essential function that is normally served by sleep. This year, we discovered that sleep deprivation is lethal in rats because host defenses against endogenous pathogens break down, allowing opportunistic, toxigenic organisms into the bloodstream. We suspected that the lethal agent had to be toxigenic for several reasons: (1) there was a lack of systematic morphological and histopathological changes, (2) the moribund state was not accompanied by seizures, convulsions or diarrhea that are indicative of specific organ-system dysfunction, and (3) sleep can prevent the mortality and readily reverse the pathology without evidence of permanent damage, whereas neurologic damage, for example, would not be expected to

recover quickly. No fever occurred in spite of the microbe invasion; temperature, which was evaluated every 30-seconds throughout the 24-hours of each day, decreased to hypothermic levels, suggesting impaired inflammatory responses. The most likely explanation for the unexplained hypothermia during a state of increased heat production might be a decrease in vascular resistance, as is found in human bacteremia-related hypothermia. Future directions of this work include investigation of cytokine mediation of the pathophysiology of prolonged wakefulness. Cytokines are known for their potentially highly catabolic and deleterious effects. Presumably, cytokines would be activated days or weeks before bacteremia became manifested, and therefore, might have mediated sleep-deprivation pathophysiology.

A role of sleep in immunocompetence has been presumed but never proven. Supportive evidence for such a role comes from strong associative relationships between sleep and changes in immune function parameters. Establishing an active role of sleep in immune function has been difficult because sleep might simply be an accompaniment of health rather than an essential restorative process. For example, animals that sleep and recover after microbial challenge might not be as sick as those that do not sleep much and do not recover. We plan to investigate whether sleep is a necessary part of the acute phase response to infection and whether it is responsible for recovery of impaired immunocompetence that was caused by sleep deprivation. For example, if sleep is the only variable manipulated in an animal that developed bacteremia as a consequence of sleep deprivation, and that animal survives, the first strong causal direction between sleep and restoration of an impaired function might be provided.

In spite of the life-threatening state caused by sleep deprivation, there is an absence of significant findings in clinical chemistry and hematology parameters, except for plasma alkaline phosphatase (ALP). ALP increases early and progressively during sleep deprivation. Changes in ALP are associated with various disease states, such as bone or liver disease. ALP isoenzyme type may provide an important clue to the nature of the early metabolic changes during sustained wakefulness. Our previous analyses suggested that the isoenzyme was not of intestinal origin. In ongoing analyses we are measuring the enzymes osteocalcin and 5'-nucleotidase in previously collected blood specimens to assess whether the ALP increase during sleep deprivation is due to bone turnover or liver enzyme changes. Future inquiries in light of the host defense findings above include an evaluation of whether the ALP increase signifies early macrophage activation (i.e., neutrophilic ALP).

Even short-term sleep deprivation impairs performance in humans, and we assume that subtle organic changes in the brain are responsible for this. However, the effects of sleep deprivation on the neurophysiology of the brain are not well-understood. To determine whether brain metabolism is affected, we have been investigating brain glucose utilization. Based on our initial analyses, brain glucose utilization in sleep-deprived rats is near-normal, but tends to be decreased overall by nearly 10%. Brain temperature, however, is increased, although not to febrile levels, indicating an unusual dissociation between brain metabolism and temperature. These changes are coupled to a dramatic increase in whole-body energy metabolism and eventual development of hypothermia. We are currently completing brain temperature analyses and autoradiography of additional experimental animals from experiments conducted this year. The future direction of this work will be to determine (1) whether the tendency of decreased brain glucose utilization reflects an increase use of alternate substrates (e.g., ketone bodies) by the brain (because body and dietary fat utilization would be expected to be increased during the whole-body hypermetabolism, and would therefore compete with glucose utilization) and (2) whether brain blood flow is decreased. In the latter case, for example, blood might not perfuse and cool the brain normally during sleep deprivation and this might account for the increased temperature of the brain during sleep deprivation. It is these types of changes that we can measure in animals that can potentially explain pathogenicities in humans, as well.

The thyroid axis is modified by prolonged sleep deprivation. This change in thyroid function might mediate the unexplained and striking increase in whole-body energy

expenditure. This year, we ran additional experiments challenging the thyroid axis with thyroid-releasing hormone (TRH) to determine peripheral responses of thyroxine and triiodothyronine (the most metabolically potent form). Nonaugmented basal and stimulated thyroid-stimulating hormone (TSH), and low plasma T_4, indicate one or more of the following: hypothalamic hypothyroidism, decreased TSH-stimulated thyroidal release, and increased negative feedback to TSH mechanisms by T_3, rather than T_4. Furthermore, low plasma concentration of T_4 would be expected to decrease the predominant peripheral conversion enzyme, type I 5'-deiodinase (5'D-I), and increase the central nervous system conversion enzyme, type II 5'D-II. This could effectively keep the brain in a euthyroid state, and therefore preserve the homeostasis of the central nervous system while the body is undergoing dynamic change. We are planning three experiments to determine whether: (1) the TSH produced by the pituitary is bioactive, and therefore, capable of stimulating thyroid production and release, (2) conversion of T_4 to T_3 is increased (as a potential mediator of catabolism), and (3) there is a tissue specific metabolic response during sleep deprivation; e.g., increased local conversion in the brain. These studies require double-labeling *in vivo* with radioactive tracers of T_4 and T_3 and dual measurement of activity and concentration of these hormones and metabolites by high performance liquid chromatography (HPLC). We are currently working to establish the HPLC technique to measure hormonal concentrations in small, radioactive specimens of blood and tissue.

There are numerous implications of new findings in sleep research for biomedicine and psychiatry. Sleep physiology and sleep function involve many aspects of neurophysiology, the immune system, hormonal systems, and brain and body temperature and metabolism. Furthermore, changes in sleep appear to play an important role in the pathophysiologies of depression and mania. To understand its clinical implications for higher-order function, we must first study sleep directly, both to determine how its effects are mediated and how physiological changes are induced by sustained wakefulness.

Formal and Functional Effects of Psychoactive Drug Treatment on the Hamster Circadian System

Wallace C. Duncan, Jr., Ph.D.

Depression is often accompanied by disturbances of the daily sleep-wake and body temperature rhythms. The facts that these rhythms are controlled by a circadian clock and that these disturbances are corrected by antidepressant drugs raises the possibility that these drugs alter the function of one or more key elements of the system that regulates circadian rhythms. Furthermore, the fact that non-pharmacological manipulations of the circadian system, such as sleep deprivation and phase advance of the sleep-wake cycle, improve depression suggests that the drugs' effects on the circadian system could be a mechanism of their therapeutic effect.

The key elements of the circadian system are 1) a neural pathway from the retina to the circadian clock that mediates effects of light on the clock, 2) the circadian clock, which is located in the suprachiasmatic nucleus of the hypothalamus, and 3) multiple effector systems that are controlled by, and in some cases provide feedback to, the circadian clock. The research described in the following sections was designed to investigate whether psychotropic drugs act on one or more of these elements.

Project Z01 MH 02430-05 CP describes effects of psychotropic drugs on the first major element of the circadian system, the retinohypothalamic tract (RHT). The RHT conveys photic information to the circadian clock that is located in the hypothalamus. The monoamine oxidase inhibitor, clorgyline, decreases sensitivity of the clock's entrainment response to light, an effect that might be mediated by the RHT. The decrease in sensitivity

may also be related to the therapeutic properties of clorgyline. Project Z01 MH 02294-08 describes effects of psychotropic drugs on the second major element of the circadian system, the circadian clock, which is located in the suprachiasmatic nucleus (SCN). The SCN generates and conveys a circadian signal to the circadian system. Previously we found that clorgyline slows the circadian clock. Since clorgyline significantly elevates and phase-delays the rhythm of serotonin (5HT) in the SCN, its ability to slow the circadian clock may depend on the drug's capacity to alter serotonergic input to the clock in the SCN. Recently we found that clorgyline delays the rhythms of body temperature, corticosteroids and melatonin. The phase-delaying property of clorgyline may be related to drug effects on the circadian clock. Finally, projects Z01 MH 02294-08 and Z01 MH 02405-06 explore effects of psychotropic drugs on the third major element(s) of the circadian system, the pacemaker-driven, effector systems that ultimately control the expression of behaviors, hormones, physiology and, through feedback effects, the circadian clock itself. For example, 5HT may alter the daily pattern of body temperature through feedback effects on the circadian clock or by its influence on effector systems (e.g. corticosteroids) that mediate the daily pattern of body temperature. These feedback effects can be examined by selective chemical lesions of serotonergic terminals in the SCN, or, since grafted SCN tissue is often not innervated by 5HT fibers, by the use of SCN graft techniques, as described in project Z01 MH 02617-01. In the following sections, effects of antidepressant drugs on these different elements of the circadian system in Syrian hamsters are described in more detail.

Psychoactive Drug Effects on the Daily Variation of Brain Temperature and Monoamines

We hypothesize that the therapeutic mechanism of antidepressant drugs depends on their effects on specific elements of the circadian system. This hypothesis is being examined by testing the effects of antidepressant and neuroleptic drugs on the daily rhythms of motor activity, temperature and sleep, as well as on monoamine levels within the SCN, the location of the circadian clock.

1. Daily Variation in Brain Temperature: During the past year this research has focused on two major areas. One area includes the effects of chronic psychoactive drug treatment on regulation of brain temperature. These experiments indicate that chronic antidepressant drug treatment with clorgyline, fluoxetine or lithium, lowers hypothalamic temperature, particularly during the rest phase of the circadian cycle. In contrast, chronic treatment with the neuroleptic drugs chlorpromazine or haloperidol increases hypothalamic temperature. Further research is required to understand the mechanisms through which these drugs alter hypothalamic temperature. For example, it is possible that antidepressant drugs decrease the set-point of hypothalamic temperature, possibly through their serotonergic properties. These drugs, through multiple effects on circulating hormones and neurotransmitters, could also alter hypothalamic temperature by changing blood flow to the hypothalamus. In depressed patients, elevated body temperature is often observed during the rest (sleep) phase, and pharmacological and non-pharmacological treatments of depression have been reported to lower body temperature. Therefore, the findings that antidepressant drugs decrease hypothalamic temperature may be important in understanding their therapeutic mechanism, and possibly the etiology of affective illness.

2. Daily Variation in Brain Serotonin and Dopamine: In light of the effects of antidepressant drugs on the circadian variation of hypothalamic temperature, a second area of research includes the chronic effects of clorgyline (an MAOI that chronically decreases hypothalamic temperature in hamsters and delays the circadian pacemaker) on brain monoamines in discrete brain nuclei reported to be involved in circadian regulation of behavior and thermoregulation. The findings indicate that chronic clorgyline treatment elevates serotonin (5HT) levels in the terminal regions of the hypothalamus (suprachiasmatic nucleus, paraventricular nucleus, and medial preoptic area) and in the thalamus (lateral geniculate nucleus), and in the cell bodies of the dorsal and median raphe. Clorgyline also elevates dopamine levels in the ventral tegmental area, the caudate nucleus

and in the nuleus accumbens. Futhermore, in all regions except the dorsal raphe nucleus and the suprachiasmatic nucleus, clorgyline treatment eliminated the circadian rhythm of 5HT that is normally present in these areas.

In the future, our studies will be extended to tricyclic antidepressants in order to determine if our observations can be generalized to other commonly used antidepressants. Our current hypothesis is that the elevation of central 5HT levels by antidepressant drugs mediates their effects on hypothalamic temperature. Since clorgyline induces large and sustained changes in 5HT levels in the dorsal raphe and the suprachiasmatic nucleus, and since these nuclei help to regulate the circadian variation of temperature, it will be important to investigate the effects of other antidepressant drugs on 5HT levels in these areas. The use of selective 5HT agonists and antagonists will be used to identify 5HT receptors that are involved in the regulation of hypothalamic temperature.

Endocrine, Behavioral and Autonomic Consequences of Chronic Clorgyline Treatment

Antidepressant drugs are reported to decrease the resting metabolic rate and increase the body mass of depressed patients. Increased body mass may be partly related to the decrease in metabolic rate. The change in metabolism that follows chronic antidepressant treatment may be related to the behavior-activating effects of the drugs. This project investigates the functional (e.g. thermoregulatory) and endocrine aspects of altered body mass. Previously, we found that chronic inhibition of type A monoamine oxidase (MAO) in Syrian hamsters with the antidepressant drug clorgyline a) prevents normal weight gain, b) decreases body lipid content, c), decreases oxygen and food consumption, and d) decreases the level of peritoneal and brain temperature. We also found that chronic clorgyline treatment alters adrenal, kidney, testis and brown adipose tissue (BAT) masses. These findings suggest that clorgyline-treatment may have significant endocrine effects. In light of the organ data, as well as reports that melatonin and corticosteroids affect thermoregulation, we examined the daily variation of cortisol, corticosterone, ACTH and melatonin in clorgyline-treated hamsters in order to assess their possible roles as mediators of clorgyline's effects on thermoregulation.

1. Effects of Clorgyline on the Daily Variation of Corticosteroids and Melatonin: Chronic clorgyline treatment was found to elevate the level, and expand the peak durations, of pineal melatonin, serum ACTH, cortisol and corticosterone. The observation that chronic clorgyline decreases the mass of BAT and elevates the levels of corticosteroids is consistent with reports that chronic corticosterone administration decreases BAT non-shivering thermogenesis (NST). In contrast, although melatonin is reported to activate NST in BAT, elevation of melatonin levels is not associated with increased BAT mass. Thus, in Syrian hamsters, chronic antidepressant drug treatment with the MAOI clorgyline may alter thermoregulation via corticosteroid-mediated inhibition of non-shivering thermogenesis.

Since chronic clorgyline treatment of Syrian hamsters elevates plasma corticosterone, cortisol and ACTH, these hormones may have important effects on thermoregulation. We found (see Z01 MH 02294-08 CP) that clorgyline chronically elevates 5HT in the paraventricular nucleus (PVN) of the hypothalamus, a nucleus that contains the corticotropin-releasing hormone (CRH) cell bodies that regulate the hypothalamic-pituitary-adrenal axis. If corticosteroids do mediate some of the thermoregulatory effects of chronic antidepressant drug treatment in hamsters, then it may be possible to examine the effects of corticosteroids on brain temperature during chronic corticosteroid or CRH antibody administration. In order to understand effects of drugs on corticosteroid regulation, it will be important to further examine the regulation of ACTH and corticosteroid release by 5HT, as well as by selective 5HT agonists. In addition, to more fully understand clorgyline's effects on corticosteroid regulation, we will measure CRH levels in the PVN during clorgyline treatment.

2. Effects of Clorgyline on the Daily Variation of Behavioral and Autonomic Thermoregulation: In collaboration with Dr. Christopher Gordon, we explored effects of clorgyline on autonomic and behavioral aspects of thermoregulation in Syrian hamsters. At cold ambient temperatures, clorgyline elevated metabolic rate and motor activity, possibly to compensate for the loss of insulative body fat and/or diminished NST. When placed on a thermal gradient for 24 hours, clorgyline-treated hamsters tended to select cooler ambient temperatures, and the usually present circadian rhythm in thermal preference was absent. Drug-treated hamsters also spent less time in the thermoneutral portion of the thermocline, suggesting that these animals' capacity to thermoregulate was less finely tuned than control animals.

The observation that between 15-30°C, the *autonomic* responses of clorgyline and saline-treated hamsters were not different, but that their *behavioral* thermal preference was different, suggests that in Syrian hamsters, clorgyline's predominant effect may be on *behavioral* thermoregulation. In low environmental temperatures, perhaps the use of behavioral thermoregulatory effectors may be increased in order to compensate for the diminished capacity of autonomic thermoregulatory effectors. The observations that chronic corticosteroid-treatment diminishes thermogenesis by BAT, and that chronic clorgyline-treatment increases corticosteroid levels, suggest that the drug's effects on hormones, particularly corticosteroids, could be related to its effects on thermoregulatory function in Syrian hamsters.

Effects of Antidepressant Drugs on Phase-Resetting of the Daily Clock by Light

In this project, we investigate the physiological properties of the neural pathways that mediate the effect of light on the timing of daily and seasonal biological rhythms. The timing of these rhythms is regulated by a circadian clock located in the suprachiasmatic nucleus. Exposure to light resets the timing of the circadian clock and the pattern of behaviors controlled by the clock. There are two aspects of this research. First, we examine the clock phase-resetting properties of light-pulses of different intensities and different durations in drug-free animals. Second, we examine the effects of chronic, antidepressant drug treatments on the clock phase-resetting properties of light.

Our recent investigations have yielded three major findings. First, we confirmed that in drug-free hamsters, the photic sensitivity of the phase-resetting response is constant throughout the circadian cycle. That is, equivalent quantities of light delivered at different circadian phases produce phase-shifts that are 50% of the maximum response at each phase. Second, we demonstrated that chronic treatment with the antidepressant drug clorgyline decreases sensitivity to light. The magnitude of the decreased sensitivity to light is dependent upon the phase of the circadian cycle at which light is delivered. More light is required to produce a 50% phase-shift response to evening light than to produce a 50% response to morning light. The shift in the daily sensitivity to light may contribute to the antidepressant properties of clorgyline. The mechanism of this shift in sensitivity is currently being examined, (for example, the drug may alter the integration of light over time). Finally, although previously published reports indicate that lithium alters visual responses, as measured by electrooculographic and neurophysiological techniques, our recent data suggests that lithium fails to alter the sensitivity or responsiveness of the photic entrainment pathway.

The results described here support previous interpretations that the photic system that is responsible for the entrainment of circadian rhythms is separate from other visual systems. For example, the photic system mediating entrainment has a high response threshold (about 6 log units above the threshold for vision), and integrates light stimuli for long periods (up to 45 minutes in contrast to 1-3 seconds for some rod photoreceptors). The facts that lithium does not alter the entrainment responses to light although the visual response is changed, and the MAOI, clorgyline does alter the sensitivity of the entrainment

response to light, suggests that this specific and distinct photic system ought to be targeted for further research.

Restoration of Circadian Function by SCN Grafts: A Tool to Explore Circadian System Organization

Daily biological rhythms are controlled by a biological clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus. Lesions of the SCN abolish circadian rhythms in motor activity, wheel-running, cortisol, melatonin, and body temperature. Further evidence that the SCN contains the biological clock is provided by the observation that fetal SCN tissue grafts can restore circadian rhythms of wheel-running in SCN-lesioned host animals. Since the fetal SCN graft restores both the *circadian oscillation* and the *behavior* driven by the oscillation, not only the clock, but also new connections between the clock and sites directly controlling the measured behavior, must have been established. The purpose of this project is to investigate a) which rhythms (e.g. body temperature, hormonal, behavioral) are restored by SCN grafts and b) the time course of the restoration.

Previous experiments have focused on a single clock-driven process (e.g. wheel-running activity or the sleep-wake cycle) to assess the success of the SCN graft. In our experiments, we will simultaneously measure multiple (behavioral, physiological, and neuroendocrine) rhythms of distinct origin, in order to determine the extent to which SCN grafts restore outputs of the circadian system (e.g. wheel-running, brain temperature, and melatonin). In early SCN graft experiments, there was difficulty distinguishing between the restoration of a circadian oscillation by donor tissue, and re-expression of the host circadian oscillation as a result of an incomplete SCN lesion. Since the circadian period of the heterozygous "tau mutant hamster" has an abnormally short period of twenty-two hours, we will transplant SCN tissue from the tau mutant hamster into SCN-lesioned wild-type hamsters. The expression of a twenty-two hour rhythm will be used as a marker of a successful SCN graft, since it can easily be distinguished from a residual twenty-four hour rhythm from the host. This experiment may help to elucidate the role of SCN efferents in the restoration of circadian rhythmicity by SCN grafts. In addition, since many successful SCN grafts restore rhythmicity in the absence of serotonergic innervation, the grafting technique will be useful in examining the role of serotonergic projections to the SCN in mediating drug effects on the circadian clock. We hypothesize that if clorgyline's effects on 5HT within the SCN are critical to slowing the daily biological clock, then clorgyline's effect on the clock will be absent in a graft that lacks serotonergic innervation.

In the past year, we have developed the methodology necessary to continuously and simultaneously monitor circadian rhythms in body temperature, motor activity, urinary corticosteroids, and urinary melatonin, in an individual hamster. This methodology will be applied in the next year to investigate the functional restoration of circadian rhythms in SCN-lesioned hamsters that have received SCN grafts.

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October 1, 1991 through September 30, 1992

Dennis L. Murphy, M.D., Chief

The Laboratory of Clinical Science is comprised of four Sections which are devoted to basic and clinical neuroscience research. A former fifth section, the Section on Comparative Studies of Brain and Behavior, (Acting Chief, Thomas R. Insel, M.D.) was administratively transferred to the Laboratory of Neurophysiology in the last year, to consolidate all of the NIMH-IRP research programs located at the NIHAC-Poolesville research center in one Laboratory. The scope of activities in the Laboratory of Clinical Science extends from analytical chemistry and neurotoxicology, through basic biochemistry, endocrinology and pharmacology, to clinical psychobiological studies of Alzheimer's disease, AIDS dementia, geriatric depression, and obsessive-compulsive disorder. An overview of the highly productive research activities in the Laboratory is provided in the brief summaries of each Section's work presented below.

Section on Analytical Biochemistry

Sanford P. Markey, Ph.D., Chief

This section develops and applies new analytical instrumentation to problems in neuropharmacology, especially to problems of neurotoxicity. Research on the analytical utility of several types of mass spectrometers and their respective sample inlets has continued with the development of liquid chromatographic-mass spectrometric assays using a particle beam interface, and the construction and testing of an external electrospray ion source for an ion cyclotron resonance spectrometer. These research tools are being applied in two areas of neurotoxicology: immunologically stimulated metabolic processes and their regulation; and oxidative damage to DNA as a mechanism of cell damage common to drugs and

infectious diseases. The former research has concentrated on quinolinic acid (QUIN), an excitotoxic intermediary metabolite of tryptophan metabolism; and the latter on thymine glycol, an oxidation product of cellular DNA thymine, as an indicator of free radical insult.

Following stimulation of the immune system, indoleamine-2,3-dioxygenase (IDO) activity in extra-hepatic tissues increases. The result is accelerated metabolism of L-tryptophan through the kynurenine pathway, leading to enhanced production of QUIN. The role of QUIN as an excitotoxin in human neurodegenerative disorders has been the subject of continued investigations by Dr. Melvyn Heyes. When applied to neurons in the central nervous system, QUIN activates N-methyl-D-aspartate (NMDA) receptors, increases neuronal firing rates, and, in high concentrations, causes convulsions and neurodegeneration. Significantly, immune stimulation in mice also increases QUIN levels in the brain which raises the possibility that neurologic deficits and neurodegeneration that occurs in certain inflammatory diseases may result from accumulation of QUIN in brain. Following our earlier work which demonstrated increased QUIN concentrations in cerebrospinal fluid (CSF) in patients with human immunodeficiency virus (HIV), we have found that these increases begin soon after seroconversion and that the highest CSF QUIN levels are found in patients with the AIDS dementia complex. Importantly, significant correlations were found between objective measures of neuropsychologic deficits and CSF QUIN levels while treatment with azidovudine (AZT) reduced CSF QUIN levels in parallel with clinical neurologic improvement. Increases in QUIN reflect the degree of immune activation of interferon-gamma. Following intracerebral immune activation, particularly where there is infiltration of the brain by macrophages, the activities of IDO and other metabolic enzymes in the kynurenine pathway are increased as determined in studies by Dr. Kuniaki Saito. Mice or gerbils given systemic injections of cytokines produce a response analogous to human systemic immune stimulation, as in septicemia. Ischemia in gerbils and poliovirus in macaques present paradigms where the immune stimulus is restricted to the central nervous system. Antibodies to interferon-gamma can attenuate QUIN formation following systemic immune activation. Following intracerebral immune activation, IDO expression is localized in glial cells and macrophage infiltrates. These studies have been paralleled by studies of cultured cells, which have demonstrated that IDO activity can be induced in macrophages with interferon-gamma. The kinetics of

kynurenine formation from deuterium or ^{13}C-labeled tryptophan have been the subject of studies by Drs. R. Boni and K. Saito.

Oxidative damage to neuronal DNA, either as a result of infectious, biochemical or environmental agents, has been postulated in the pathogenesis of neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. To examine this hypothesis, we have been developing mass spectrometric methods for the quantification of damaged DNA bases, and have selected thymine glycol as an initial target. Thymine glycol is formed by the action of oxygen free radicals on thymine in DNA, and is normally removed and replaced with thymine by restriction endonuclease repair enzymes. If neuronal DNA is damaged beyond the capacity of repair, DNA isolated from the brain tissue of aged patients would show greater thymine glycol concentration than that from matched controls. We have used the base catalyzed hydrolysis and borohydride reduction of DNA-bound thymine glycol to release 2-methylglyceric acid as a specific product which can be quantified by gas chromatography-mass spectrometry. Experimental methods have been tested to maximize the sensitivity of an gas chromatographic-mass spectrometric assay. This assay has been used to demonstrate that the level of thymine glycol in 'undamaged' DNA is on the order of 4 per million thymine residues. When DNA is exposed to various types of crystalline silica known to cause varying degrees of mutagenesis, there is a correlation between DNA breaks as determined on gels and the levels of thymine glycol measured. The assay of thymine glycol is being applied to DNA from cultured cells exposed to varying oxidative insults to determine whether damage and repair can be quantified.

Developments in mass spectrometric methodology has been in three areas: Fourier transform ion cyclotron resonance spectrometry (FT-ICR); improved derivatization procedures for either liquid chromatography-particle beam -mass spectrometry, or gas chromatography-mass spectrometry; and in a collaborative project on organic ion imaging Dr. Carl Ijames has designed, assembled and tested an external atmospheric pressure electrospray ion source and ion transport lens assembly for the FT-ICR. The high resolution mass analyses of high molecular weight (kilodalton) proteins and DNA fragments would be possible with such an instrument. The efficient transport and mass analysis of thermally generated ions was first demonstrated. Success has now been realized for the detection and analysis of ions electrosprayed from aqueous solutions

at atmospheric pressure and transported into the high vacuum region of the FT-ICR. However, electrospray ion currents from macromolecules remain difficult to optimize, and additional instrumentation is required to effectively utilize this instrument as a routine analytical device. Dr. Riccardo Boni has applied sensitive negative chemical ionization mass spectrometric detection to derivatized tryptophan and kynurenine pathway metabolites eluting from a liquid chromatographic column into a particle beam interface, and has used these methods to quantify the production of 13-carbon labeled kynurenine from 13-C labeled tryptophan by various strains of cultured cells responding to gamma-interferon. Studies aimed at improved derivatization reagents for gas or liquid chromatography mass spectrometry were directed toward testing of a new compound, pentafluorophenylboronic acid, which reacts with bi-functional organic molecules such as cis-diols and catechols to form pentafluorophenylboronate esters. Mr. Simpson has tested several analytes, and excellent gas chromatographic and electron ionization mass spectrometric properties were observed. However, the desired sensitivity enhancement from electron capture negative chemical ionization was found only with those substrates which can stabilize the resulting gas phase anion. Thus, additional structural derivatives will be investigated in order to improve detection limits for analytes of interest. At Oak Ridge National Laboratory, Dr. Peter Todd has been developing an organic ion imaging mass spectrometer based upon instrumentation initially assembled in this laboratory. His group has demonstrated the ability to spatially resolve ions derived from an organic surface of one cm diameter. Initial tests with several compounds of interest to this laboratory (epinephrine, metabolites of MPTP) have demonstrated the potential of such instrumentation to directly image organic molecules. Current collaborative efforts are directed toward imaging these species in tissue slices or cultured cells, a non-trivial extension of the technique because of the severe problems of sample charging and the fact that cellular structural elements may mask the presence of entrained organic compounds.

Section on Clinical Neuropharmacology

Dennis L. Murphy, M.D., Chief

This Section explores the biochemical and behavioral pharmacology of novel as well as some standard psychoactive agents in attempts to understand both how these drugs work and, more importantly, how these drugs can be used to investigate normal and abnormal brain neurochemistry and neuropsychologic function. Our recent studies can be subdivided into four main areas of investigation: (1) a geriatric psychopharmacology research program focused on patients with Alzheimer's patients and elderly patients with depressive disorders; (2) an allied program of neuropsychological investigations of Alzheimer's disease, AIDS dementia and other neuropsychiatric disorders; (3) psychopharmacological and psychobiological studies of patients with obsessive-compulsive disorder (OCD); and (4) laboratory studies directed, in particular, towards studies of serotonin-selective drugs and their receptors and signal transduction mechanisms, and towards studies of a cell culture model for Alzheimer's disease.

The Unit on Geriatric Psychopharmacology, headed by Dr. Trey Sunderland, has concentrated on the underlying biology and treatment of Alzheimer's disease and geriatric depression. Of particular interest in the last year has been the continued development of a nasal epithelial cell culture model to study the underlying cause of Alzheimer's disease. Specifically, cells from the nasal cavity of living Alzheimer patients have been obtained and successfully propagated in the laboratory. These dividing cells show many characteristics of brain neurons and offer a unique opportunity to directly test the processing of amyloid and other cellular mechanisms in Alzheimer's versus normal elderly subjects. Not only do these investigations have possible diagnostic implications but they also provide a potential model for the neurochemical changes accompanying Alzheimer's disease.

On the clinical front, the Unit is proceeding with a number of new drug studies. The medication studies with scopolamine and other "challenge" drugs are designed to establish a better pharmacologic model of the memory deficit associated with Alzheimer's disease, while others

are more directly related to potential therapies. Therapeutically, it is our goal to establish a method to combine the modest effects of multiple medications to create a more effective overall treatment strategy (e.g., physostigmine plus deprenyl). This combination approach has proven successful in other major medical illnesses such as cancer and may well prove useful with the dementias. Already in our first pilot study, we have learned that these medications can be given together safely in demented subjects. Together with the basic science studies, these clinical projects afford a broadly-based program in Alzheimer's disease which is aimed at understanding the cause of the illness as well as toward the development of new therapies. As for the clinical studies with older depressives, we are just now completing a successful project with high-dose deprenyl in treatment-resistant depressives. We are also continuing to compare the biochemical and clinical profiles of our Alzheimer and depressed subjects to better understand the overlap in behavioral symptoms between these two populations.

The Cognitive Studies Unit headed by Dr. Alex Martin, has continued to pursue two related lines of research. (1) to develop and test models of cognition via study of the way specific processes break down following brain injury or disease; (2) to evaluate the cognitive status of psychiatric patients in order to test hypotheses concerning possible neuroanatomic correlates of these disorders. This Unit also works in close collaboration with the Unit on Geriatric Psychopharmacology by providing clinical neuropsychological evaluation to aid in patient screening and diagnosis, detailed baseline cognitive evaluation for correlational studies with biological parameters, and tasks and procedures for use in therapeutic drug trials and drug challenge studies.

The unit has completed a series of studies on the ability of Alzheimer's patients to learn and remember objects and spatial patterns by contrasting performance under explicit recall conditions with indirect or implicit measures of memory. These studies have indicated that, under certain conditions, Alzheimer's patients can show normal implicit learning. This finding suggests that some form of cognitively-mediated memory may be preserved even in patients with wide-spread limbic and cortical pathology. Interestingly, this appears to be true for pictures of real objects, but not for novel, spatial configurations. In fact, not only

Alzheimer's patients, but normal elderly and young controls failed to show evidence of implicit learning of spatial information, even though the normal subjects could explicitly recognize the previously presented spatial patterns. These findings suggest that implicit learning may be dependent on the use of material that is amenable to a structural object description. A related series of studies have addressed the question of whether the object naming deficit in patients with Alzheimer's disease is due to a loss of knowledge versus impaired retrieval from an intact knowledge store. Using semantic priming paradigms, evidence has been obtained in support of a model that posits that damage to posterior regions of the temporal lobe in the disease results in an actual loss of degradation of the semantic representations of objects. These degraded representations are, in turn, proposed to be responsible for impaired naming and other types of word-finding problems in patients with Alzheimer's disease.

In collaboration with investigators at the Walter Reed Medical center, the Unit has continued its studies of the neurobehavioral consequences of early Human Immunodeficiency Virus infection. This longitudinal investigation has continued to document subtle psychomotor and cognitive slowing, impaired motor-skill learning and other deficits consistent with involvement of subcortical regions in a subgroup of early stage HIV-infected individuals. Moreover, these motor and cognitive deficits were unrelated to anxiety and depression and significantly correlated with the concentration of quinolinic acid in cerebral spinal fluid as measured by Dr. Melvyn Heyes and colleagues in the Laboratory of Clinical Science, Section on Analytical Biochemistry.

Based on the apparent sensitivity of reaction time measures and motor-skill learning tasks to subcortical dysfunction, a study was designed in collaboration with Dr. Teresa Pigott and colleagues in the Unit on Adult OCD Studies to evaluate performance of unmedicated adult OCD patients. Contrary to our expectations, no differences were found between the OCD patients, patients with trichotillomania, and normal controls. Taken together with the results of the HIV study, these data suggest that the types of deficits seen in patients with Huntington's disease and other disorders of the basal ganglia may provide a good model for HIV-related cognitive dysfunction, but not for adult OCD.

The adult obsessive-compulsive disorder (OCD) research progress is now headed by Dr. Margaret Altemus, following Dr. Teresa Pigott's departure to Georgetown University in July, 1992. In an exploration of possible overlap between OCD psychopathology and the psychopathology of the eating disorders, significantly more disturbed eating attitudes and behavior were found in OCD patients of both sexes than controls, contributing to a growing body of data indicating both biological and psychological similarities between these syndromes which may be of therapeutic relevance.

Cerebrospinal fluid studies of OCD patients compared to controls revealed enhanced secretion of the stress-responsive neuropeptides arginine vasopressin, somatostatin, and corticotropin-releasing hormone in the patients. Enhanced secretion of these neuropeptides may play a role in the pathophysiology of OCD since in animals these peptides promote vigilance and also delay extinction of conditioned behaviors. Subsequently, Dr. Altemus demonstrated that antidepressant medications which are effective treatments for OCD are distinguished by an ability to decrease hypothalamic synthesis and secretion of vasopressin in animal model studies, suggesting that development of other pharmacologic agents which could directly reduce vasopressin activity should be explored as a possible new treatment for this illness.

Laboratory studies of serotonin-selective agents in rodents by Dr. Charanjit Aulakh and Dr. Peter Lesch continued to provide very valuable comparative data relevant to the group's clinical studies. By using various 5-HT receptor subtype selective antagonists, m-chlorophenylpiperazine (m-CPP, a 5-HT_1 agonist)-induced prolactin secretion was found to be mediated by stimulation of postsynaptic 5-HT_{1C} receptors while corticosterone secretion induced by m-CPP may be mediated by an antagonistic effect at 5-HT_3 receptors or by non-serotonergic mechanisms. The food intake suppressant effect of the hallucinogenic agent, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) was demonstrated to be mediated by stimulation of both 5-HT_{1C} and 5-HT_2 receptors. In another study, we have demonstrated that clonidine stimulates growth hormone secretion by activation of α_2-heteroreceptors

present on 5-HT nerve terminals which, in turn, enhance 5-HT activity by stimulation of post synaptic 5-HT_{1C} receptors to promote growth hormone releasing factor. Furthermore, either 5-HT_{1C} receptors or α2-adrenergic heteroreceptors or both are functionally subsensitive in the Fawn-Hooded rat strain (an animal model for depression) relative to the Wistar rat strain.

In a separate series of experiments, chronic treatment with the tricyclic antidepressants and clorgyline (a monoamine oxidase type A inhibiting antidepressant) decreased the steady state concentrations of G protein α subunit Gsα and to a lesser extent Giα in several brain regions, while Goα was increased by tricyclics but not clorgyline. Chronic treatment with carbamazepine decreased Gsα in several brain areas reaching significance in the neostriatum, while chronic lithium treatment had no unequivocal effect. Lithium treatment significantly increased Giα in the hypothalamus and hippocampus, whereas carbamazepine decreased Giα in the frontal cortex. These findings indicate that long-term treatment with antidepressant and antibipolar drugs exert differential effects on G protein a subunits, and that antidepressant or antibipolar efficacy may potentially be based on functional modifications of signal transduction.

Section on Histopharmacology

David M. Jacobowitz, Ph.D., Chief

This laboratory has focused on calretinin, a central nervous system neuron specific calcium binding protein that we have isolated and purified. We have taken a multidisciplinary approach in order to reveal as much as possible concerning (1) the localization by histochemical means, (2) quantitative studies by radioimmunoassays, and (3) functional studies by utilizing a phosphorylation system within rat brain membranes. This year, we have added molecular biological studies with the isolation of a calretinin cDNA clone from a lgt11 rat brain library.

The following calretinin studies have been completed or are in progress:

A detailed mapping of calretinin positive cells and fibers in the rat thalamus was completed using *in situ* hybridization histochemistry and immunocytochemistry. Results of this study revealed populations of calretinin positive cells in several discrete thalamic nuclei (e.g., reticular, rhomboid, reuniens, paraventricular) and in regions which overlapped defined nuclear thalamic boundaries (e.g., cells in the central and intralaminar nuclei continuous with the central grey). Unilateral cochlea ablations were found to increase the immunoreactivity of neurons in the ipsilateral ventral cochlear nucleus and contralateral trapezoid body. However, no changes were seen in mRNA label. *In situ* hybridization histochemistry revealed that calretinin mRNA is present early in development (E9) in chick embryos (Winsky).

A 39 kDa protein, whose phosphorylation is inhibited by calretinin, was found predominantly in mitochondrial membranes and was localized in several peripheral tissues with greatest amounts in the testis. Calretinin inhibited the phosphorylation of this protein in all regions and subcellular fractions where the 39 kDa band was visible on autoradiograms. Divalent cations stimulated both the phosphorylation of the 39 kDa (Mg^{2+}, Mn^{2+}, Ni^{2+}, Ca^{2+} or Co^{2+}) and the inhibition by calretinin (Mg^{2+}, Co^{2+} or Ni^{2+}). Zn^{2+} inhibited both the phosphorylation of the 39 kDa band and the effect on calretinin, while EDTA attenuated the calretinin effect. Calretinin also produced a slight attenuation in the phosphorylation of a 44 kDa band identified as the alpha subunit of pyruvate dehydrogenase. In contrast, initial examination of the effects of a related calcium binding protein (calbindin D-28k) revealed a stimulation of protein phosphorylation and a reversal of the inhibitory effect of calretinin on phosphorylation of the 39 kDa protein. This suggests a possible functional role of calretinin in modifying mitochondrial enzyme activity (Winsky).

In order to explore the role of calretinin in the brain and its regulation at the molecular level, we have cloned and analyzed the rat cDNA coding for calretinin. An immunoreactive clone was isolated from a rat brain cDNA expression library in lgt 11. The 1.45 kb insert was

subcloned into the Eco RI site of the pGEM-4Z transcription vector for further analysis. Its identity was confirmed by comparison with human calretinin. The rat cDNA sequence comprised a 54 bp 5' untranslated region, an 816 bp open reading frame including start and stop codons, and a 579 bp 3' untranslated region. The open reading frame has 271 codons coding for a putative protein of 31.4 kDa. A polyadenylation signal and 13 adenylate residues were found near the 3' end. The evolutionarily conserved calcium binding domains and connecting regions and the limited changes observed between rat and chick primary structure lead us to believe that calretinin interacts with other highly conserved constituents of brain cells. This claretinin cDNA clone provides a new probe for the analysis of specific neurons in the central nervous system. The cDNA probe will allow a more detailed analysis of calretinin expression in the brain and will be useful for screening genomic libraries for the complete chromosomal gene (Strauss).

Uncontrolled Ca^{2+} flux is thought to orchestrate cell death due to excitatory amino acids. Studies suggest a relationship between calcium binding proteins (CaBPs) and resistance to excitotoxicity. It is not clear, however, if neurons survive due to an absolute resistance to the excitotoxin. For example, in chick retina, NMDA kills most amacrine and some ganglion cells, whereas other retinal populations are unaffected. The resistance of neurons in the outer layers of retina is due to the absence of NMDA receptors on these cells. Amacrine neurons in retina are ideal to study the relationship between CaBPs and excitotoxicity because most amacrines are sensitive to NMDA in a dose dependent manner and the CaBPs, parvalbumin, (PV), calretinin, (CR), and calbindin (CB) are found in this layer. Exposure of embryonic day 19 chick retina for 60 min to either 25, 100, 250 or 500 µM NMDA caused a dose dependent increase in LDH release measured after 24 hr of recovery. At 24 hr, retina was fixed and processed for PV, CR and CB immunoreactivity. The number of amacrine cells positive for the CaBPs were counted and correlated to LDH release. Statistical analysis showed a negative correlation between NMDA mediated LDH release and loss of PV + amacrines and no correlation with loss of CR or CB + amacrines. Thus, as LDH increased, the number of PV cells, but not CB or CR cells declined. Exposure to 500 µM NMDA for 24 hr resulted in a near total loss of PV and CB and 66% of CR + amacrines.

These data suggest that CB and CR positive amacrine cells show a relative resistance to NMDA (Zeevalk).

The possible coexistence of calretinin with other calcium binding proteins, parvalbumin and calbindin D28k, and with GABA, was studied in non-pyramidal cells of the rat dorsal hippocampal formation. The majority of the calretinin-containing neurons (83%) were found to be immunoreactive for GABA (79%) in the dentate gyrus, 84% in the CA2-3, and 88% in the CA1 subfield). Analysis of the calretinin-immunoreactive cells of these subfields revealed that the two morphologically distinct types of calretinin neurons, i.e., the spiny and the spine-free cells, differ in their immunoreactivity for GABA. The overwhelming majority (92%) of the spine-free neurons were GABA-positive, whereas the immunoreactivity of spiny cells was ambiguous. At the sensitivity threshold of the immunocytochemical techniques used in the present study, most of the spiny cells (89%) had to be considered as GABA-negative. Colchicine treatment resulted in a degeneration of calretinin-immunoreactive neurons; therefore, its effect on the GABA content of spiny neurons could not be evaluated. Nevertheless, the observations suggest that calretinin-containing neurons are heterogeneous both morphologically and neurochemically. Examination of the coexistence of calcium binding proteins revealed that none of the hippocampal cells contained both calretinin and parvalbumin in any regions of the hippocampus. Some overlap was detected between the calretinin- and the calbindin-containing cell populations, 5.1% of the former and 6.2% of the latter were immunoreactive for both calcium binding proteins. This may be due to a small degree of cross reactivity with calretinin. Thus, these results demonstrate that the majority of calretinin-immunoreactive neurons are GABAergic and represent a subpopulation of non-pyramidal cells with no or only a negligible overlap with the subpopulations containing the other calcium binding proteins, parvalbumin and calbindin (Freund).

Section on Pharmacology

Juan M. Saavedra, M.D., Chief

The general interest of the section is the study of the role of hormones, neuropeptides and biogenic amines on the central regulation of the sympathetic, endocrine, cardiovascular and immune systems, on biological rhythms, and on brain development. Currently the section is focused on the role of angiotensin II, and in particular, on that of its receptor subtypes.

Dr. Keisuke Tsutsumi continued his study on development and characterization of brain angiotensin receptor subtypes. He advanced the characterization of the brain AT_2 receptor subtypes which he had recently discovered. Dr. Tsutsumi found that binding of angiotensin II to the AT_{2A} receptors in brain was sensitive to reducing agents, such as dithiothreitol, whereas binding of ANG II to the AT_{2B} receptors was insensitive. These observations further indicate that the two receptor subtypes may be biochemically distinct. The anatomical selectivity of the AT_{2A} and AT_{2B} receptors further suggest different physiological roles.

Novel sites of localization for AT_{2B} receptors were found by Dr. Tsutsumi in the deep cerebellar nuclei and the cranial nerve nuclei. These findings support the initial hypothesis of a physiological role of AT_2 receptors in central motor function.

In addition Dr. Tsutsumi found that all angiotensin II receptor subtypes were present in the rat fetal brain at 18 days of gestation, with characteristics similar to those of the adult rat. This indicated a possible role for the receptor subtypes during embryonic development.

Dr. Tsutsumi also conducted experiments in peripheral tissues. Dr. Tsutsumi found that AT_2 receptors predominated in the fetal kidney, whereas the adult organ contained almost exclusively AT_1 receptors. These findings indicated that AT_2 receptors may play important roles in the developing kidney.

Upon Dr. Tsutsumi's return to his native country in January 1992, Drs. Frank Heemskerk and Gladys Ciuffo continued his work in the characterization of brain and peripheral AT_2 receptors. Dr. Heemskerk has demonstrated that a new radiolabelled ligand, [^{125}I]-CGP 42112A, can label brain and adrenal AT_2 receptors selectively. Dr. Ciuffo has successfully solubilized and cross-linked AT_1 receptors from adult rat kidney, and solubilized AT_2 receptors from newborn rat kidney. These experiments will allow her to proceed with the purification of the selective receptor subtypes, in an effort to study their biochemical characteristics and development.

Drs. Christer Strömberg and Liisa Naveri set up in our laboratory the laser-doppler method for continuous measurement of cortical cerebral blood flow in the rat. Using this method, they demonstrated dramatic changes in cerebrovascular blood flow after administration of the AT_1 blocker losartan, the AT_2 displacer PD 123177, and the stimulation of AT_2 receptors with an angiotensin II infusion in the presence of losartan. The results indicate that cerebrovascular AT_2 receptors, originally described in our laboratory may play important roles in the regulation of blood flow to the brain. Pharmacological manipulation of these receptors, resulting in large differences in cerebrovascular resistance, is now possible. These findings may have relevance for the treatment of cerebrovascular disorders.

Dr. Stefan Zorad obtained the cDNA for the AT_{1A} angiotensin receptor from Dr. Inagami's Laboratory at the Vanderbilt University and a cDNA fragment was subcloned into the pGEM plasmid in order to prepare a riboprobe to be used in *in situ* hybridization studies of the AT_1 receptor. This work allows Dr. Zorad to begin to study the localization, regulation and metabolism of AT_1 receptors in selected brain areas and peripheral tissues under a great variety of experimental conditions.

In another series of experiments, Dr. Zorad, in collaboration with Dr. Strömberg, set up a method for isolation of brain cortical arterioles of 6 to 80 μm in diameter, which are very important in the control of cerebrovascular resistance. Drs. Zorad and Strömberg demonstrated that this preparation contains angiotensin II binding sites, and established

optimum binding conditions. These experiments will allow the characterization of receptor subtypes and signal transduction mechanisms in brain arterioles. Together with the *in vivo* experiments in cerebral blood flow, these studies could clarify the mechanism of action of angiotensin II in the regulation of cerebrovascular autoregulation.

Dr. Alicia Seltzer continued her studies on the regulation of brain angiotensin II receptors involved in the gonadotropic control. Dr. Seltzer found that in the median eminence, which contained exclusively AT_1 receptors, angiotensin II was able to stimulate phosphatidylinositol turnover. These observations strongly suggest that AT_1 binding sites in the median eminence may be considered physiologically active receptors, with phosphatidylinositol turnover as one of their signal transduction mechanisms.

Dr. Mohan Viswanathan continued his studies on the developmental aspects of angiotensin II receptor subtypes, and their relation to growth. Dr. Viswanathan found that after experimental wound healing, the number of AT_2 receptors increased in subcutaneous tissues, and that these receptors were localized in a collagen-rich band. These data indicate that AT_2 receptors could be involved in the process of wound healing and tissue repair.

Dr. Viswanathan, in collaboration with Dr. Strömberg set up the method of balloon angioplasty in rat aorta. Loss of endothelium results in the formation of neointima, and in this tissue, by quantitative autoradiography, Drs. Viswanathan and Strömberg demonstrated increased expression of AT_1 receptors. The possible role of AT_1 receptors in neointima formation opens new possibilities for the understanding of mechanisms of repair and growth in peripheral arteries.

Dr. Marisabel Torres is conducting a study of the detailed developmental expression of angiotensin II receptor subtypes in the rat brain. Dr. Torres has found that the crucial time for the dramatic decrease in the expression of brainstem AT_2 receptors is between 1 and 4 weeks postnatally. This information can be useful to better understand the role of AT_2 receptors during postnatal development.

Dr. Kathleen M. Michels continued her studies on the regulation of pituitary IGF receptors by female reproductive hormones. Dr. Michels found that estrogen administration modulated components of the IGF system, including IGF itself and the IGF binding protein, in the anterior pituitary. Dr. Michels also found that optic nerve transection upregulates components of the IGF system in the visual pathway of the rat.

Dr. Juan M. Saavedra initiated a study of the effects of blockade of AT_1 receptors on the response to stress. He found that AT_1 blockade with losartan partially inhibited the ACTH release during immobilization stress in the rat, supporting the hypothesis of a role for angiotensin II, and in particular, AT_1 receptors, during stress.

Annual Report for the Child Psychiatry Branch
National Institute of Mental Health
October 1, 1991 - September 30, 1992
Judith Rapoport, M.D., Chief

The Child Psychiatry Branch conducts research on biological aspects of child psychiatry. Brain imaging techniques and response to pharmacological agents are major tools for this research. However, clinical, genetic and family, and endocrinological and immunological studies are ongoing.

Within the National Institute of Mental Health (NIMH), there are ongoing collaborations with: the Laboratory of Psychology and Psychopathology (LPP) (Dr. Zahn); the Laboratory of Clinical Science (LCS) (Drs. Murphy and Potter); the Laboratory of Cerebral Metabolism (LCM) (Drs. Zametkin and Cohen); and the Clinical Neuroscience Branch (NSB) (Dr. Pickar). Collaborations within ADAMHA are ongoing: the National Institute on Alcohol Abuse and Alcoholism (NIAA) (Dr. Brown). Collaborations are also taking place with other National Institutes of Health (NIH) institutes: National Institute of Aging (NIA) (Dr. Stanley Rapoport and staff); National Institute of Neurological Disorders and Stroke (NINDS) (Drs. Grafman, Hallett, Schifman, Dr. Parker); the National Institute on Deafness and other Communication Disorders (DCD) (Dr. Ludlow, Ms. Pikus); and the NIH Clinical Center (Drs. Grothe, Barnett, and Leitman). Collaborations with other Institutions include: Brown University (Dr. Louise Kiessling); University of California, Los Angeles (Drs. Robert F. Asarnow and Rachelle Caplan); University of Michigan (Dr. John Fink); University of Pennsylvania (Dr. Alan Fiske); the Johns Hopkins University (Dr. Ann Pulver); Western Carolina Center (Drs. Crawford, Bodfish, and Madison); Carnegie Mellon (Dr. Jonathan Cohen); Nathan Kline Institute (Dr. Cooper); and Tufts University (Dr. Feldman).

Research is organized into four broad interrelated areas:

1. Brain imaging studies of developmental disabilities (dyslexia, autism) with both Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MRI) are being carried out. The pattern of localized dysfunction with PET and abnormal symmetry with MRI seen in dyslexia are consistent with the scattered reports of psychopathology. These data are being compared with brain imaging studies (coordinated by this unit) across the disorders discussed below. In addition to building our brain imaging library across disorders, we have recruited additional manpower to deepen our technical and theoretical approach to brain imaging in developmental psychopathology.

2. Studies of obsessive compulsive disorder and related disorders have grown to include the first systematic studies of trichotillomania, a disorder of compulsive hair pulling that affects as many as two million women in this country, and studies of stuttering, a possible compulsive behavior. A follow-up study of children with obsessive compulsive disorder (OCD) is demonstrating the close relationship of OCD to Tourette's Disorder. In addition, Sydenham's chorea is being explored as a neuroimmunological model for OCD. Studies of response of both

childhood autism and severe chronic stutterers suggest that both these disorders may improve selectively on the serotonin uptake inhibitor, clomipramine which has selective benefit for OCD. This work has broadened clinical perspective on unwanted, repetitive, stereotyped behaviors not typically classified as OCD or as anxiety disorders, and suggested new treatments.

3. Studies on aggressive and hyperactive children have focused on comparison of methylphenidate and dextroamphetamine and on the treatment of children with both hyperactivity and motor tics or Tourettes. Pilot data shows that the short-term worsening of motor tics with methylphenidate (but not amphetamine) may disappear with higher doses and/or continued treatment. The operation of a self-contained day treatment program has let us observe these children closely in a protected environment and demonstrate the safety and efficacy of a controversial treatment that could not have been studied elsewhere. An ongoing study will examine the predictive ability of CSF 5-HIAA, the principal serotonin metabolite to predict poor outcome in a replication sample of young hyperactive children.

4. A new inpatient study of children with schizophrenia has already shown that patients, age 8-16 years, can be found with presentation identical to that seen in adult onset schizophrenia. Pilot data indicates that familial, autonomic and brain imaging patterns resemble those seen with the adult disorder. This study will be the major new thrust of the Child Psychiatry Branch over the next five to eight years.

Summary Annual Report

Clinical Neurogenetics Branch, FY 1992

Elliot S. Gershon, M.D., Chief

Overview:

The research of the Clinical Neurogenetics Branch is aimed at advancement of knowledge in the areas of inherited neuropsychiatric disease, genetic variation in behavior and in genes expressed in brain, and genomic organization. In the Section on Clinical Genetics, the goals are discovery of genetic causes of vulnerability to major psychiatric illness and related disorders, progress in genetic mapping of common diseases, and genomic analysis of specific genes of importance in the nervous system. Our investigative strategies include clinical diagnostic studies of major psychiatric disorders in families, systematic genomic mapping for susceptibility genes in psychiatric illness, theoretical and applied studies of genetic linkage analysis in complex disorders, and genomic analysis and identification of disease mutations in proven and candidate disease genes. In the Unit on Genomics, the goals are development of integrated transcriptional maps of large chromosome regions, molecular genetic analysis of brain development, and molecular genetic and biochemical studies on cyclophilins.

Section on Clinical Genetics:

Psychiatric disorders pedigrees: [Dr. Gershon, Dr. Gejman, Dr. Ram, Dr. Robb, Ms. Guroff, Mrs. Kazuba and Mrs. Maxwell]. Our series of 20 moderate-sized bipolar manic-depressive families for systematic genomic mapping of illness, which to our knowledge is the largest multiple-pedigree collection of its kind, was completed in 1991, with 338 of our cultured cell lines employed in systematic mapping. Other bipolar pedigree collection efforts continue: bipolar pedigrees from the Sephardic Jewish population in Israel, and participation in the NIMH collaborative study on Diagnostic Centers for Psychiatric Linkage Studies.

In that NIMH collaborative study, two interview instruments have been prepared for bipolar (BP) and schizophrenia (SZ) families: the Diagnostic Instrument for Genetic Studies (DIGS), and the Family Interview for Genetic Studies (FIGS). A reliability study has been submitted for publication. Collection of our Branch's pedigree series for this study has begun with 4 families collected.

We are continuing to extend our schizophrenia pedigree collection, and have begun a panic disorder pedigree collection, with 19 families studied so far.

Systematic genomic mapping for a susceptibility locus in manic-depressive illness: [Drs. Detera-Wadleigh, Gejman, Gershon and Berrettini (Jefferson Medical College)]. This is a multiyear project, in which suitably spaced marker loci from each chromosome are genotyped in each person in a

series of pedigrees, and linkage analyses are then performed, including analyses which can detect linkage in the presence of genetic heterogeneity. We have so far published results on 136 marker loci on chromosomes 1, 5, 10q, 11q, 13, 15, 17, and X. We have unpublished results on an additional 169 marker loci. No linkage has been found, so far. It is the nature of a project like this one that most of the information developed is an exclusion map, that is, a map of the locations on which a disease locus is unlikely to be found.

We are now doing mainly microsatellite marker loci, because of their improvement in informativeness over RFLPs. Multiplexing methods have been developed for PCR and for electrophoresis.

Statistical power of genetic linkage analysis: [Drs. Goldin, Martinez and Gershon]. There are numerous unresolved statistical issues in linkage detection in diseases with complex inheritance (that is, diseases which do not fit simple dominant or recessive inheritance). We have computed the power to detect linkage under various two-locus models of inheritance in pedigrees and nuclear families and compared methods of linkage analysis. Linkage can generally be detected for epistatic models if the linked locus is dominant and the unlinked locus either dominant or recessive. For an additive model, linkage is more difficult to detect. The power to detect linkage, when the true model is two-locus, is not substantially better when the data are analyzed under a two-locus model vs. a single locus model although the estimates of the recombination fraction are better.

Pedigree data on linkage of Alzheimer's disease to chromosome 21 and 19 markers are being analyzed as part of the Genetic Analysis Workshop 8. We are attempting to better localize the gene on chromosome 21 that causes early onset Alzheimer's in some families, by carrying out linkage analysis under more complex models of age-dependent penetrance.

Efficiency of linkage calculations for the bipolar mapping project is improving by the development of methods for reducing the number of alleles for marker systems with many alleles, and by implementing linkage procedures on high speed computers. We now run linkage analyses on the massive parallel processing computer recently acquired by DCRT, and at the Federal computing center in Frederick.

Detection of disease mutations: [Drs. Gejman, Ram and Gershon]. Denaturing gradient gel electrophoresis (DGGE) is being utilized to screen for mutations in candidate disease genes. In psychiatric disorders, we are scanning dopamine receptor genes.

In proliferative disorders, we have studied GTPase activating protein (GAP), and familial multiple endocrine neoplasia type I (FMENI). GAP is involved in the regulation of normal *ras* proteins through its catalytic domain, and plays a role in the signal transduction pathway of some growth factors. We screened several tumor types for mutations in GAP. We have found mutations in the SH2 region of GAP, which regulates signal transduction, in basal cell carcinomas.

In FEMNI, deletions are known to occur on chromosome 11q13. In collaboration with investigators in NIDDK, we have compared alleles in blood with alleles in tumor, to detect allelic loss as a result of microdeletion. 58 percent of the tumors from patients with FMENI, and 26 percent of sporadic parathyroid tumors, exhibited allelic loss with at least one marker from chromosome 11. As a result of these findings, the region of deletion is better defined than previously: FMENI gene was located within 7.5cM between PGA centromerically and INT2 telomerically.

A new method of scanning DNA fragments for mutations spectrophotometrically is being developed.

Steroid-retinoic acid receptor gene superfamily: [Dr. Detera-Wadleigh] Human glucocorticoid receptor (hGR) promoter fragments, subcloned into a vector with luciferase reporter gene, were transfected into GR-expressing cell lines to investigate basal transcription activity by transient expression assays. A region with the greatest effect on transcription was identified by systematic deletions of promoter segments. Down-regulation by dexamethasone using the promoter constructs tested so far has not shown impressive repression. Gel mobility shift assays reveal that the putative regulatory sequences that have been tested so far bind nuclear factors in a specific manner.

Genomic clones encoding some regions of the human mineralocorticoid receptor (hMR) were isolated to establish the gene structure of the receptor and determine the exon/intron sequences flanking the splice sites.

Unit on Genomics [Drs. Brennan and Hochgeschwender]:

Integrated Transcriptional Maps of Human Chromosomes: We have developed and applied methods for systematically identifying transcribed sequences from large, arrayed genomic libraries. We have identified a large fraction of the genes on chromosome 21 expressed in the adult cerebral cortex. Analysis of these clones is important for the understanding of Down syndrome, and the methods are of general importance to the problem of isolating disease genes.

Molecular Genetic Analysis of Brain Development: In our systematic study of the gene regulation underlying brain development, we have identified six novel genes which are coordinately regulated during cerebellum development. They define a new regulatory pattern: they are expressed at high levels in late embryonic stages, and are decreased or turned off in the adult. These genes are candidates for analysis by mutation in embryonic stem cells.

Isolation and Characterization of Conserved Eukaryotic Genes: The study of conserved genes in the model eukaryote *Saccharomyces cerevisiae* is a new approach. We have used this approach to identify the first essential function for a cyclophilin, the receptor for the commonly used immunosuppressant cyclosporin A. Further, we have developed a recombination based protocol for the systematic selection of other conserved genes.

Awards

Elliot S. Gershon, M.D.: (Chief, Clinical Neurogenetics Branch)

Samuel W. Hamilton Award, American Psychopathological Association, 1992

National Alliance for the Mentally Ill (NAMI) Exemplary Psychiatrist Award, 1992

Department of Health and Human Service, Public Health Service, The Outstanding Service Medal, February 21, 1992

Adelaide S. Robb, M.D.: (Medical Staff Fellow, Unit on Genomics, CNG)

NARSAD Young Investigator Award

ANNUAL REPORT SUMMARY
CLINICAL NEUROSCIENCE BRANCH
Edward I. Ginns, MD, PhD, Acting Chief

I. Research Highlights

The reorganization of the Clinical Neuroscience Branch was completed during the past year and is now comprised of the Sections on Molecular Neurogenetics and Molecular Pharmacology. The research within the Branch focuses on:

a) the characterization of ligand binding and gating properties of the GABA receptor complex;
b) the study of neurotrophic and neuroprotective phenomena;
c) the study of inbred and genetically engineered strains of mice that are models of inherited disorders;
d) the development of therapeutic approaches utilizing cell transplantation and gene therapy.
e) the molecular genetic mechanisms underlying the clinical heterogeneity of inherited disorders affecting the nervous system; and,
f) systematic mapping of the genome for genes responsible for manic-depressive illness in the Amish;

The identification of genes responsible for bipolar illness through systematic mapping of the human genome is a major goal of both Section's, and is being undertaken with Dr. Janice Egeland (Amish Study at Hershey, Pennsylvania, of the University of Miami), Dr. Tim Keith (Collaborative Research, Inc.) and Dr. David Pauls (Yale University). The Branch's clinical investigation of these disorders is closely linked to its basic research interests, and is directed toward the eventual development of new diagnostic tests and therapies.

II. Section on Molecular Neurogenetics

The research with the Section is directed toward elucidation of the genetic and biochemical mechanisms that are responsible for inheritable disorders affecting the nervous system, so that diagnostic tests and more efficient therapy can be developed. Of particular interest are manic-depressive illness in the Amish and lysosomal disorders, such as Gaucher disease.

The baculovirus (insect cell) expression system has proven very useful for the recombinant production of proteins, including human and mouse glucocerebrosidase and human saprosin. In particular, we have studied those parameters that are critical to the scale-up of expression in both shaker flasks and bioreactors for the large scale production of enzyme for human replacement studies. During the past year as part of our CRADA with Enzon, Inc., we have explored the polyethylene glycol (PEG) modification of human

glucocerebrosidase. The PEG modification of glucocerebrosidase should give the enzyme a longer serum survival and permit either subcutaneous or intramuscular, rather than intravenous, administration. Clinical trials in Gaucher patients of this modified glucocerebrosidase are planned for next year.

In order to provide larger quantities of recombinantly produced proteins, transgenic animals are being studied for their potential as bioreactors. Dr. Sidransky has been studying the mammary gland specific promoters and other elements that affect the levels of expression of human glucocerebrosidase in the milk of mice, and ultimately pigs, during lactation. This work is being performed in collaboration with Dr. Bob Wall (USDA), Dr. Lothar Hennighausen (NIDDK) and Dr. Heiner Westphal (NICHD).

In an effort to better identify those Gaucher patients that might benefit from early intervention, we have directed our identification of the mutations in the glucocerebrosidase gene toward phenotype-genotype correlations. Dr. Sidransky has reviewed a large number of clinical histories of Gaucher patients and our findings indicate that there are exceptions to the published generalizations of other groups. The mechanisms responsible for the phenotypic heterogeneity in Gaucher patients are still not well understood and work continues to physically map and describe the gene locus on chromosome 1q21.

Dr. Brian Martin work's on the identification of the primary and tertiary structures of proteins, using microsequencing techniques has included enzymes, channel blocking toxins, and a thermal regulatory peptide from the lizard. Dr. Martin's work includes numerous collaborations including proteins such as a phospholipase from scorpion venom, yeast transketolase, an a rat angiotensin receptor. In addition, his group continues to develop novel methodologies for protein structure analysis, including a more convenient quantitative method for identifying phosphorylated residues during amino acid sequence determination.

Our transgenic mouse research has led to the first animal model of a human metabolic disease. We have generated an animal model for Gaucher disease by creating a null allele in embryonic stem cells through gene targeting and using these genetically modified cells to establish a mouse strain carrying the mutation.
This research was a collaborative effort between our Section and scientists at the Whitehead Institute for Biomedical Research and Department of Biology, MIT, the Department of Cell Biology and Genetics, Erasmus University, Rotterdam, and the Laboratory of Mammalian Genes and Development, NICHD. Mice homozygous for this mutation have less than 4% of normal glucocerebrosidase activity, die within twenty-four hours of birth and store glucocerebroside in lysosomes of cells of the reticuloendothelial system.

The irregular respiration, poor feeding and decreased movement of the mutant mice are consistent with the nervous system dysfunction responsible for the fulminant clinical course seen in the more

severely affected subset of type 2 infant Gaucher patients. An association of Gaucher disease with congenital ichthyosis in bith the human and the mouse has been particularly noteworthy. These findings have emphasized that there should be an increased awareness for Gaucher disease in the differential diagnosis of the "collodion baby" or congenital ichthyotic skin phenotypes. Mutant mice carrying less deleterious mutations should therefore be models for the more frequently observed milder presentations of Gaucher disease. The mice should be useful for studying the pathogenesis and the phenotypic diversity seen in Gaucher disease, and will provide a model in which enzyme replacement, cellular transplantation and gene transfer therapies can be evaluated.

Our gene therapy research for Gaucher disease has focused on the development of receptor mediated targeting of DNA, as well as, the transplantation of recombinantly manipulated cell lines that can function as depots of enzyme replacement and/or substrate metabolism. New retroviral constructs have been developed in collaboration with Richard Mulligan's laboratory and the use of these virus based vectors for gene therapy is being evaluated in both in vitro and in vivo experiments.

We continue the pursuit of a gene responsible for manic depression disorder. We have undertaken a systematic search of the human genome in order to identify a gene responsible for manic depressive disorder in an Old Order Amish Pedigree. The original collection of cell lines from Amish pedigree 110 has been extended. We have used over 250 markers spaced at approximately 20 centimorgans. Because of the uncertainties in ascertaining phenotype and the lack of any definitive biological marker for bipolar illness, we have performed our analyses using several different clinical hierarchies. The restrictive definition of the disease should provide the more homogeneous population for analyses. Our models have assumed a dominant gene and included age dependent penetrance. To date, no LOD scores greater than 3 have been obtained from pairwise linkage analyses. We are performing additional analyses with other models of inheritance and diagnostic hierarchies. We are expanding our collection of cell lines from both normal and affected family members and updating our diagnostic data. We are continuing our systematic screening of the genome for a gene responsible for bipolar illness using highly informative microsatellite markers.

SECTION ON MOLECULAR PHARMACOLOGY

Steven M. Paul, M.D., Chief

The research goals of the Section on Molecular Pharmacology have changed somewhat over the past year or so. While we have continued to pursue our studies on neurosteroids, and their neuroactive properties, as well as the interaction of various drugs with $GABA_A$ receptors, (see below), we have focused considerable effort in characterizing a novel neuroprotective phenomena we have recently observed in cerebellar granule cells. These findings have catalysed our interest in studying the neurotrophic and neuroprotective effects of neurotransmitters (such as GABA and glutamate) and in delineating the genetic programs induced by such trophic substances. Some of these studies are outlined below.

One of the primary research interests of our Section over the past 10 yrs has been the characterization of both the ligand binding and gating properties of the $GABA_A$ receptor complex. Our earlier work employed a facsile method developed in our laboratory for measuring GABA-activated ^{36}chloride [$^{36}Cl^-$] flux in synaptoneurosomes. Using this method we studied desensitization induced by GABA agonists and the interaction of a number of sedative-hypnotic drugs, including barbiturates and ethanol, on $GABA_A$ receptor function. Moreover, we demonstrated that two naturally-occurring steroid hormone metabolites 3α hydroxy 5α dihydroprogesterone (allopregnanolone) and tetrahydrodeoxycorticosterone (allatetrahydroDOC) were extremely potent "barbiturate-like" ligands of the $GABA_A$ receptor complex. This work has been widely replicated and has prompted recent efforts at delineating whether the steroid/$GABA_A$ receptor interaction(s) is of physiological significance. In collaboration with Dr. Robert Purdy of the Southwest Foundation for Biomedical Research in San Antonio we have developed specific and sensitive radioimmunoassays for measuring both allopregnanolone and allotetrahydroDOC. We have measured these steroids in rat and human serum as well as rat and human brain. Our data demonstrate that activation of the hypothalamic-pituitary-adrenal (HPA) axis by "stress" results in robust elevations in the serum/plasma and brain concentrations of these neuroactive steroids. Moreover, for allopregnanolone, demonstrable brain levels are measured even in adrenalectomized animals, suggesting the possibility of de novo synthesis within the central nervous system (CNS). Related work has established the structure-activity relationships for the steroid-induced modulation of $GABA_A$ receptors and has revealed several other natural steroids that are active at $GABA_A$ receptors. Several pharmaceutical companies are now attempting to develop synthetic derivatives which could prove useful as drugs for the treatment of anxiety disorders/insomnia and various seizure disorders. Our laboratory has served (and continues to serve) as a consultant to several of the companies attempting to develop such novel therapeutic agents. Our own efforts, however, have focused on defining other steroid/membrane receptor interactions that may be of pharmacological and (or) physiological significance. In this regard we have recently found that pregnenolone sulfate (the most abundant sulfate derivative of pregnenolone) is a positive allosteric modulator of the excitatory amino acid neurotransmitter glutamate, acting at NMDA receptors. The positive modulatory effects of pregnenolone sulfate is observed at concentrations between 5-100 μM and has been defined

using both electrophysiological and microspectrofluorometric methods. Pregnenolone sulfate is a relatively selective modulator of NMDA responses in much the same way that the A ring reduced progesterone metabolites modulate $GABA_A$ receptors. Given these findings we are now characterizing other steroids to see which have significant actions on excitable membranes, perhaps at other ligand-gated and voltage-dependent ion channels. We hope to address the fundamental question(s) of whether steroids are synthesized in brain and if the concentrations achieved are of physiological significance.

We have continued and extended our work on the expression of $GABA_A$ receptors in primary cultures of neurons from rat and chick. These experiments have attempted to follow up on earlier studies in which we observed changes in the level(s) of $GABA_A$ receptor α subunit mRNAs in the cerebral cortex of rats chronically administered intoxicating doses of ethanol. We have also found that exposure of fetal chick or rat cortical neurons in vitro to desensitizing concentrations of GABA results in a time-and concentration-dependent reduction in the level(s) of α subunit mRNAs. The ability of GABA to "down regulate" receptor-encoding subunit mRNAs is blocked by $GABA_A$ receptor antagonists and is observed in the absence of any change in mRNAs for a variety of housekeeping genes (i.e. β actin, tubulin, etc.). By contrast, in cerebellar granule cells (where GABA has been shown to have neurotrophic actions) exposure of neurons to GABA results in a time-and concentration-dependent "up regulation" of $GABA_A$ receptor α subunit mRNA(s). This effect appears to be blocked by GABA receptor antagonists. Thus, GABA, depending on the population of neurons examined, is capable of "up or down" regulating the synthesis of its own receptor. At present, it is unclear as to how activation of $GABA_A$ receptors results in changes in the transcription and/or processing of genes or mRNA encoding receptor subunits. We are currently studying the mechanisms underlying the effects of psychoactive drugs on $GABA_A$ receptor gene expression and the ability of GABA to alter the expression of receptor subunits in primary cultured neurons. These studies could contribute to a better understanding of the mechanisms involved in tolerance and dependence to sedative/hypnotic agents.

The ability of neurotransmitters such as GABA and glutamate to rapidly alter the excitability of neuronal membranes (by activating ligand-gated ion channels) has been recently shown to activate other second and third messenger systems and to affect the transcription of genes involved in important neuronal functions. In complimentary studies we have been exploring the factors involved in the neurotoxicity of excitatory amino acid (EAA) transmitters in defined neuronal populations. Using primary cultures of cerebellar granule cells we have characterized glutamate toxicity as being mediated predominately by the NDMA receptor subtype and being time-delayed in its full manifestation. Cerebellar granule cells are also sensitive to the chemical neurotoxin MPP^+. Paradoxically, we have observed that preexposure of cerebellar granule cells to low (subtoxic) concentrations of either NMDA or glutamate results in a robust neuroprotective state against both glutamate and MPP^+. The induction of this neuroprotective state requires a preincubation time of several hours to be fully manifest and can be blocked by specific NMDA receptor antagonists. Moreover, inhibitors of new RNA and protein synthesis completely block the neuroprotective state induced by NMDA. These results suggest that activation of NMDA receptors in cerebellar granule cells induces the expression of a neuroprotective protein. We believe these findings are

novel since they are the first to suggest that low-level stimulation of NMDA receptors can be neuroprotective to neurons whereas high concentrations of glutamate acting at NMDA receptors is neurotoxic. Moreover, they suggest the presence of an intrinsic, transcriptionally-directed, neuroprotective mechanism which is yet to be fully characterized. These findings could be important in elucidating the cellular factors that contribute to the susceptibility (or lack thereof) of certain populations of neurons to both endogenous and exogenous toxins.

Annual Report of the Experimental Therapeutics Branch
National Institute of Mental Health
October 1, 1991 through September 30, 1992
David Pickar, M.D., Chief

Report not available at time of printing.

Annual Report of the Laboratory of Developmental Psychology
National Institute of Mental Health
October 1, 1991 through September 30, 1992

Marian Radke-Yarrow, Ph.D., Chief

The goal of the research conducted in the Laboratory of Developmental Psychology is to advance knowledge concerning major mental health problems of children, with a focus on the basic processes of adaptive and maladaptive psychosocial development. A developmental orientation and a longitudinal approach are central to the Laboratory's research program.

Research generally is carried out collaboratively within the Laboratory and also with investigators from other institutes and universities. It is multi-disciplinary, involving the research interests and expertise of developmental psychology, child psychiatry, and developmental endocrinology.

Because of the Laboratory's emphasis on longitudinal research, there is considerable continuity in the program content of successive Annual Reports. The major projects are: (A). A longitudinal study of the course of development of offspring of unipolar and bipolar depressed parents and a comparison group of children of well parents. Begun when the children were two years of age, this study follows the children through childhood and into adolescence. Multiple psychological, psychiatric, and family assessments are made, as well as physical and psychobiological assessments. (B). Biological and psychological consequences of childhood sexual abuse. This multi-method longitudinal study investigates the psychological, social, physical, and endocrinological development of sexually abused girls. Intensive yearly evaluations are made. (C). Early conduct disorders. This research is also a multi-method longitudinal study that includes psychological, psychiatric, environmental, and psychobiological evaluations of hard-to-control children and a comparison group, following them from four years to seven years. (D). The psychological impact on children of exposure to chronic community violence. The nature of exposure, children's responses to violence, the role of the adults, and children's psychiatric symptoms are investigated. This study has a 2-year follow-up component although it is primarily a cross-sectional study. (E) A new research initiative that focuses on the psychological consequences of physical child abuse is now in planning and piloting stages.

More detailed descriptions of these projects are given below, with research findings and anticipated research directions.

A. The longitudinal study of offspring of depressed parents.

The data base consists of child and family assessments made at three-year intervals, of 110 families and 220 children. The data-sets on mother, father, and children consist of standard psychiatric assessments, behavioral observations of affective behavior and quality of relationships. Assessments of the children, also include cognitive measures, physical examinations, measures of peer relationships and school behavior, and measures of hypothalamic-pituitary-adrenal axis function. Measurement of EEG patterns is planned.

The research questions investigated in this study fall into six broad areas:

(1). Continuities and discontinuities in offspring development. Continuities and

discontinuities in children's development are examined in relation to parental psychiatric status and functioning in the parent role. Children of unipolar, bipolar, and well parents follow very different developmental paths. In comparisons of children of well and affectively ill parents, significant differences in prevalence rates of diagnosable psychiatric problems appear at about ages 5 to 6 years; these differences widen in later childhood. Offspring of unipolar mothers, compared with the other groups, develop problems earlier, show a higher degree of continuity over time within the individual child, and are more likely to have a sibling with problems. Offspring of bipolar mothers, compared with offspring of unipolar mothers, more often cope well until middle or late childhood. Continuity of problems is lowest in children of normal parents. Between middle and later childhood depressive affect increases in the offspring of affectively ill groups, and comorbidity also increases. (Investigators: Marian Radke-Yarrow, Pedro Martinez, Mary Beth Fox, Editha Nottelmann, Barbara Belmont). Analyses of developmental course and child outcomes are now being extended to the adolescent period. In these analyses, our objectives are to refine parental diagnostic information (severity of illness, Axis II characteristics, family history, and parenting impairments) and examine the predictive value regarding child outcomes.

(2). Individual differences in developmental course. We have selected for intensive study (a) children who are at maximum risk (in terms of both parents affectively ill, affective illness in family history, impaired parental functioning, and high family stress), but who nevertheless are developing adaptively, and (b) children from normal control families at low risk who are developing serious problems.

These children are being compared with the children who are developing according to theoretical prediction (i.e., high risk conditions leading to problem outcomes and low risk conditions leading to good outcomes). These analyses become especially interesting when children in the same family develop differently. Analyses are in process. (Investigators: Marian Radke-Yarrow, Earnestine Brown).

(3). Depression-related parenting impairments. Through observational approaches in experimental and naturalistic settings, parent-child and family interactions are examined. Many of these analyses are not yet at a stage for reporting findings. Among the issues they address are the ways in which parents' affective illness (a) appears in negative and unpredictable verbal and behavioral content in relating to their children, (b) influences parents' abilities to carry out normal socialization functions, and (c) interferes with marital relationships and family functioning. (Investigators: Barbara Belmont, Elizabeth DeMulder, Kathleen Free, Gale Germain, Pedro Martinez, Editha Nottelmann, Louisa Tarullo, and Marian Radke-Yarrow).

(4). Intergenerational transmission of affective problems. Gender differences are of special interest in families of unipolar depressed mothers, given the higher prevalence of depression in women than men. Inquiry was directed to the possible behavioral transmission of gender patterns through differing socialization experiences of male and female offspring of depressed mothers. At the youngest ages studied, depressed and well mothers differed more with respect to their treatment of daughters than their sons. Sons more than daughters coped with maternal negative affect with irritability and anger; daughters more than sons responded with anxiety and sadness. The gender pattern was reenacted in the adolescent period.

Depressed mothers and their adolescent daughters exhibited especially stressful relationships. Further investigation of gender socialization is underway.

(5). Methodological questions. Assessments of the children are obtained from multiple informants and methods, in categorical diagnostic terms and also as continuous variables. We can, therefore, examine some issues of much current concern in the field; namely, how psychiatric evaluations of children are best made, and how multiple data sources are best used and interpreted. What are the advantages/disadvantages of categorical and dimensional assessments? The several sources of information (mother, father, child) regarding children's problems are compared, taking into account the specific problem area, the psychiatric status of parent, age, gender, and problem status of the child. Data from the DICA are used in these analyses. A second methodological focus in child assessment is a comparison of psychiatric interview data with observations of child behavior in strategically chosen situations. These methodological studies are in progress.

(6). Psychobiological Studies. The hypothalamic-pituitary-adrenal axis function is being investigated in the offspring of affectively ill and well parents. Adult patients with depression show a pattern of basal hypercortisolism, associated with attenuated plasma ACTH response to ovine corticotropin-releasing hormone (oCRH). The purpose of the study is to determine whether the offspring of affectively ill (unipolar and bipolar) mothers show a similar pattern of response to CRH stimulation. Their response is compared to that of offspring of normal control mothers. A standard oCRH stimulation test is performed by administering a bolus injection of 1 mcg/kg body weight of ovine CRH at 8 PM. Plasma levels of ACTH and cortisol are measured before, during, and after oCRH administration. This study is carried out with the collaboration of Dr. George Chrousos (NICHD). Data collection has just been completed. (Laboratory investigators: Giovanna Municchi and Pedro Martinez).

B. Psychobiological Effects of Sexual Abuse

This multi-method, longitudinal study assesses the psychological, social, physical and endocrinological development of sexually abused girls and matched controls, aged 6-15 years at the first evaluation. The sample consists of 170 children, equally divided into abused and controls. Over 100 children have participated in three of the intensive yearly evaluations. The research questions investigated in this study fall into three broad areas:

(1). Differences in psychological and social development in sexually abused compared to non-abused children. Social, emotional, and cognitive development are tracked with a variety of standard measures. Recent results include findings that sexually abused girls differ significantly on locus of control measures compared to the non-abused group. Sexually abused girls also show differing friendship patterns, generally being significantly more isolated than comparison girls. Following puberty, sexually abused girls rapidly overtake the comparison girls in numbers of boyfriends, who generally are several years older than the girls. This social pattern places abused girls at considerable risk for early pregnancy, drug use, failure to complete school and antisocial behaviors. In school settings, sexually abused girls are rated by their teachers as having significantly more acting out behaviors, less frustration tolerance, poorer social skills, less task orientation and more attentional problems. Their teachers are blind to the nature and purpose of the study. A blindly-rated observational study of the non-verbal

communication styles of abused and comparison girls in the presence of a strange male demonstrated robust differences between the two groups. The higher levels of "flirtatious" behavior in the abused girls may provide an important window of therapeutic intervention for these troubled children.

(2). Differences in physical growth and development in sexually abused and non-abused girls. Sexually abused girls have significant differences in cortisol levels and regulation compared to non-abused comparison subjects. They show significantly higher morning cortisol levels and lower afternoon levels. Comparison of the area under a 40 minute curve obtained by drawing three cortisol samples (0, 20 & 40 minutes) reveals that the sexually abused girls have significantly greater reactivity to mild stressors than the comparison subjects.

(3). Differences in the developmental course of potentially pathological processes in sexually abused and non-abused girls. Based on structured psychiatric interviews, sexually abused girls have significantly higher rates of depression, attention deficit disorder, and conduct disorder diagnoses compared to matched non-abused comparison subjects. Sexually abused girls score significantly higher on a measure of dissociation (Child Dissociative Checklist) developed in LDP. Approximately 20% of the abused girls score in the pathological range associated with dissociative disorders. However, measures of hypnotizability do not differ significantly between these groups, providing additional evidence that hypnotizability differs from clinical dissociation. High levels of dissociation are strongly correlated with aggression and sexualized behavior in the abused girls. In both samples, dissociation in the child was significantly positively correlated with dissociation in the mother, providing the first empirical evidence of an intergenerational link for this process. (Laboratory investigators: Frank Putnam, Barbara Everett, Karin Helmers).

C. Children at Risk for Conduct Disorders

Children of preschool age who are presenting out-of-control, aggressive, hard-to-manage behavior are investigated as children at risk for developing later disruptive behavior disorders. In an initial follow-up study of antisocial behavior of two year olds, considerable continuity was found in their antisocial behaviors when they were reassessed at beginning school age. A more comprehensive, multi-assessment study is now underway of four-year-olds classified as at high, moderate, and low risk (based on parent and teacher reports). Broad and intensive assessments are made: (a) cognitive batteries to detect possible verbal and nonverbal disabilities and difficulties in behavioral control and attention, (b) social-cognitive batteries including measures of empathy, and symbolic representations of problem solutions, and (c) procedures for assessing emotion regulation under conditions of disappointment, frustration, and temptation, with particular interest in anger regulation. Psychophysiological changes in heart rate, vocal tone, and skin conductance as a function of exposure to specific emotion stimuli are also measured. Parent-child interactions and parental socialization practices and family life stresses are examined.

In follow-up assessments at 6 to 7 years of age, child and family assessments are being repeated with additional child measures that include physical examinations, assessment of cortisol levels, intelligence tests, social competence at school, and psychiatric interviews.

In preliminary analyses of Time 1 data, several patterns are discernible. (1) Contrary to expectation, aggressive children are responsive to distress in other individuals. Their capacities for empathy do not appear to be muted, but these children sometimes show overarousal. Moreover, empathy occurs in the broader context of dysregulated negative emotions. Compared with controls, children at risk display more negative emotion and less positive emotion, and engage in more disruptive behaviors in the structured situations designed to challenge emotion regulation. In symbolic representations, children at risk focus on aggressive, rather than prosocial, solutions to interpersonal problems, including representations of chaotic, disorganized, and brutal violence, sometimes drawn from television and film themes. In the cognitive domain, overall IQ does not distinguish children at risk from controls but certain specific aspects of cognitive function have a better than chance ability to identify children at risk. These aspects are verbal skills (fluency, comprehension), perceptual skills (visual analysis, visual perception), and executive skills (ability to delay response, to solve problems flexibly).

Longitudinal analyses will focus on characteristics of the children, in conjunction with family dynamics and parent-child interaction patterns, to determine which of the children at risk continue to have serious problems and which children move toward more adaptive development. (Laboratory investigators: Carolyn Zahn-Waxler, Pamela M. Cole, and Giovanna Municchi. Collaborating investigators from other institutes include: Marku Linnoila and Gerald Brown, NIAAA, and Steven Suomi, NICHD.

D. Effects of Community Violence on Children's Emotional and Social Development.

An interview survey was conducted with children and parents living in an inner-city very low income area that has a high crime rate. Children with very high exposure and those in the same neighborhoods with low exposure to violence were selected for detailed individual psychiatric assessment. A structured psychiatric interview was developed specifically to probe for children's responses to various kinds of violence. Also, an instrument was constructed, using a cartoon format, to elicit responses from children with limited language skills. The cartoons depict reactions that are characteristic of post-traumatic stress symptoms. The child is asked to indicate the kinds of thoughts, feelings, and behaviors that (s)he experiences. This procedure has been used successfully with these highly stressed children. It is used along with the clinician's unstructured interview.

Among these children, 14% of 1st- and 2nd- graders and 22% of 5th- and 6th- graders had witnessed severe crimes. Seventy-three percent of 1st- and 2nd- graders had witnessed drug transactions. Children with high exposure were studied further. Based on psychiatric interviews, clinically significant symptoms of maladjustment were present in approximately a third of the children. High exposure to violence was associated with symptoms of anxiety, depression, intrusive thoughts, impulsivity, and feelings that they might not live very long.

Other research activities

There are research activities of our staff that do not directly involve projects initiated or supported by the Laboratory but to which our staff contribute. These include the following: Dr.Putnam has directed a seven center study of dissociation in psychiatric patients, using a questionnaire measure that he developed. He is also collaborating in large scale studies of the biological correlates of dissociation in eating disorder (University of Michigan) and in post-

traumatic stress disorder (National Center for PTSD, West Haven, CT) patients. Carolyn Zahn-Waxler is a collaborator in a longitudinal behavior-genetics study of twins (University of Colorado). Mz and dz twins are compared to examine genetic influences on early emotional expressions. Individual differences in empathy and guilt during the second year of life show modest genetic influence, with increasing evidence for the role of socialization. Emotions of anger and fear show stronger genetic influence and weaker environmental influence. Dr. Pedro Martinez is a collaborator with investigators in NIDDKD in a study of Attention Deficit Hyperactivity Disorder and altered cerebral glucose metabolism.

Professional/scientific activities of the Staff.

Laboratory staff serve as editors, members of editorial boards, and reviewers for major scientific journals. Carolyn Zahn-Waxler this year has been appointed editor of Developmental Psychology, and member of the Council of Editors, APA.

Laboratory staff members have been active in organizing and reporting on two research conferences in the past year. One was a conference of editors of journals in psychiatry and psychology. It had the objective of furthering research-relevant communication between the two disciplines, and heightening awareness of interdisciplinary issues that influence research. A second conference brought together leaders in research in the field of conduct disorders of children. Papers prepared for the conference will be published as a special issue of a journal. Both conferences were jointly conducted by the Child and Adolescent Disorder Research Branch (Peter Jensen and John Richters) and the Laboratory.

Dr. Frank Putnam and Dr. Carolyn Zahn-Waxler were the recipients of honor awards. Dr. Waxler received the Administrator's Award for Meritorious Achievement, and Dr. Putnam received the Commendation Metal, Commissioned Corps Award.

The Laboratory received supplemental support for research and conferences from the Childhood Transitions Network of the John D. and Catherine T. MacArthur Foundation.

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Mortimer Mishkin, Ph.D., Chief

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Two cortical visual pathways

Cortical tissue essential for visual perception extends far beyond the striate cortex to include not only the prestriate regions of the occipital lobe but also large portions of the temporal and parietal lobes. Converging evidence from our neurobehavioral and neurobiological studies indicates that these extrastriate regions contain numerous areas that can be distinguished both structurally and functionally. Moreover, the multiple visual areas appear to be organized hierarchically into two separate cortical visual pathways or streams, each having the striate cortex as the source of its initial input.

One stream consists of an occipitotemporal projection system, interconnecting the striate, prestriate, and inferior temporal areas. This stream is critical for the visual recognition of objects, and its further projections to limbic structures in the temporal lobe and ventral portions of the frontal lobe allow the cognitive association of visual objects with other stimuli and other events, such as emotions and motor acts. The other visual stream consists of an occipitoparietal projection system, interconnecting the striate, prestriate, and inferior parietal areas. This stream is critical for visual spatial perception, and its further projections to both dorsal limbic and dorsal prefrontal cortex enable the construction of cognitive maps, as well as the visual guidance of motor acts that may have been triggered initially by activity in the occipitotemporal stream.

To trace the flow of feedforward and feedback information through each of the two cortical visual streams or pathways in detail, we have undertaken a series of anatomical studies using both anterograde and retrograde tracing techniques (e.g., amino-acid autoradiography, horseradish peroxidase histochemistry, and axonal transport of fluorescent dyes) in combination with electrophysiological recording. Our goal in these studies is to identify the multiple visual areas that comprise this cortex, delineate their topographic organization, and explore the complex circuitry of their interconnections.

Our results indicate that the occipitotemporal pathway begins with the striate projection to the second and third visual areas, V2 and V3, which in turn project to area V4. These three prestriate areas are arranged in adjacent belts that nearly surround the striate cortex, and, like the striate cortex, each belt contains a representation of the contralateral visual field.

Area V2 corresponds to cytoarchitectonic area OB, while V3 and V4 together correspond to area OA, exclusive of its dorsal part. The major output of V4 is to a widespread region within the inferior temporal (IT) cortex. Within posterior IT cortex, or architectonic area TEO, label was found primarily after V4 injections involving the representation of the central visual field, whereas within anterior IT cortex, or architectonic area TE, label was found after injections of any part of V4. Thus, mainly central field representations in V4 project to TEO, while both central and peripheral field representations in V4 project to TE.

Physiological studies have shown that TE has no discernible visuotopic organization. Rather, neurons in TE have very large receptive fields that nearly always include the center of gaze and frequently cross the vertical meridian into the ipsilateral visual field. Thus, a single neuron in TE can "see" an object no matter where it occurs in the field, which is in keeping with the crucial role this area plays in object recognition. Surprisingly, almost nothing is known about the properties of neurons in TEO. As a first step in studying these properties, we have mapped TEO electrophysiologically and found that it contains a crude representation of the entire contralateral visual field. An especially high percentage of receptive fields recorded in TEO include the fovea, which is consistent with the input this area receives from the central visual field representation of V4. Because lesions of TEO, unlike those of TE, produce impairments in pattern perception rather than in object recognition, a physiological comparison of TEO with TE should help in understanding the neural mechanisms underlying these functions.

In our recent studies of area TEO, we have found that this area relays to area TE visual information it receives from prestriate areas V2-V4. Because V4 also relays to TE the input it receives from V2, we investigated the distributions of V4-projecting and TEO-projecting neurons within V2. The results showed that although TEO-projecting neurons are far sparser, they are intermingled with V4-projecting neurons; both of these classes of V2 neurons are located in cytochrome-oxidase rich thin stripes and interstripe regions, i.e., those regions with high proportions of color-selective and orientation/length-selective cells, respectively. Thus, V2 appears to relay similar kinds of visual information to V4 and TEO, which could explain the partial sparing of color and form vision that is seen after lesions of V4.

Unlike the occipitotemporal pathway, the occipitoparietal pathway begins with striate, V2, and V3 projections to visual area MT, which is located in the caudal portion of the superior temporal sulcus, mainly within the dorsolateral portion of cytoarchitectonic area OA. MT projects to three additional areas located in parietal cortex, or cytoarchitectonic area PG. Thus,

although MT receives inputs from areas belonging to the occipitotemporal pathway, its outputs appear to be directed mainly into the parietal lobe.

One projection zone of MT, area VIP, lies ventrally in the anterior two-thirds of the intraparietal sulcus, while the other two, areas MST and FST, are located on the medial bank and floor, respectively, of the superior temporal sulcus (STS). To examine the role of these areas in visual function, we have recorded the electrophysiological properties of neurons within MT's projection zones and compared their properties with those of neurons in MT itself. Our results indicate that, like neurons in MT, a majority of those in MST and a third of those in FST are highly sensitive to the direction of stimulus motion but are insensitive to both the form and color of a visual stimulus. Compared to neurons in MT, however, neurons in MST and FST integrate motion information over progressively larger portions of the visual field and respond selectively to more complex types of visual motion. Thus, MT and the areas to which it projects may constitute a cortical system for motion analysis. To help identify additional components of this motion-analysis system, we have determined the targets of both MST and FST, which include not only widespread regions of the posterior parietal cortex but also several areas on the medial bank and floor of the anterior portion of the superior temporal sulcus. Thus, the cortical pathway for motion analysis seems to split into two components, a parietal and a temporal. Lesions along the parietal component of this system are known to cause impairment in spatial perception, eye movements, and visually guided hand movements, but the effects of lesions along the temporal component of the system remain to be explored. The results of our anatomical studies indicate that the motion-analysis system might influence the control of eye and head movements through two parallel subcortical channels to the cerebellum, one via the pons and another via the nucleus of the optic tract, the lateral terminal nucleus, and the inferior olivary nucleus.

Recent work has shown that a monoclonal antibody directed against a nonphosphorylated neurofilament protein, SMI32, labels pyramidal cells in monkey visual cortex in areal specific patterns. In V1, SMI32 labels the cells projecting to MT, namely, those in layer 4B and the Meynert cells in layer 6. In V2, V3, V4, and MT, SMI32-immunoreactive (ir) cells are mainly in layers 2 and 3. Area MT is unique in having a band of SMI32-ir cells in layer 6. Because SMI32 also labels alpha ganglion cells in the retina and the magnocellular cells of the lateral geniculate nucleus, SMI32 may be a selective marker for components of the magnocellular pathway through the cortex. To test this possibility, we injected distinct fluorescent tracers into areas MT and V4 and analyzed the proportion of retrogradely labeled cells that were double-labeled with SMI32. Our results indicated that, of the cells projecting to MT, about 90% of those

in V1 and 75% of those in V2 were SMI32-ir. By contrast, of the V2 cells projecting to V4, less than 50% were SMI32-ir. These preliminary results are thus consistent with the predominantly magnocellular input to area MT and the relatively equal contributions of magnocellular and parvocellular inputs to area V4. Moreover, because SMI32-ir neurons are selectively lost in Alzheimer's disease, a selective vulnerability of cells receiving magnocellular inputs may explain the impairment in visuospatial function that is characteristic even in the early stages of the disease.

We have begun to study the functional development of the motion-analysis system using the 2-deoxyglucose method. Our preliminary findings indicate that this system is not fully developed at birth, but it appears to mature slightly earlier than the occipitotemporal system for object recognition.

Although MT plays a pivotal role in the occipitoparietal pathway, it does not provide the sole route by which visual information from striate cortex reaches the parietal lobe. Other potential pathways include those through additional visual areas located in occipitoparietal cortex. Our recent findings indicate that these areas receive inputs representing predominantly the peripheral visual field, which presumably reflects the importance of such inputs for spatial vision. We have also found a predominance of peripheral over central field inputs in the projections of V4 to area TF on the parahippocampal gyrus, suggesting that this temporal lobe region, like the parietal cortex, may have chiefly visuospatial functions. By contrast, the predominance of central field inputs from V4 to TEO in the posterior portion of the inferior temporal cortex presumably reflects the importance of these inputs for pattern perception.

To determine how the object and spatial information carried by the occipitotemporal and occipitoparietal pathways are integrated to yield a unified percept, we have begun to investigate possible anatomical sites of interaction. Accordingly, we have made multiple injections of two different anatomical tracers into the lower bank of the intraparietal sulcus (following removal of the upper bank) and into the inferior temporal cortex, and then identified and compared the distributions of cells in extrastriate visual cortex projecting to these two destinations. Although cells projecting to temporal and parietal cortex were found to be located mainly in different areas, two areas contained cells projecting to both: V4 and the posterior bank and floor of the STS outside MT. In both V4 and STS, labeled cells projecting to the two destinations were intermingled, though the projection to parietal cortex was heavier from the peripheral than from the central field representation of V4, and the reverse was true for the projection to temporal cortex. The results of injecting the temporal and parietal cortex with different anterograde tracers demonstrated that V4 provides

feedforward information to both temporal and parietal cortex, whereas zones within the STS are sites for convergence of information from these two regions. We have also investigated the subcortical connections of the object-vision and spatial-vision processing pathways and have found that, like the cortical connections of these two pathways, the subcortical connections are highly segregated. Whereas parietal but not temporal cortex projects to the pons and superior colliculus, temporal but not parietal cortex is reciprocally connected with the amygdala. These results indicate, on the one hand, the importance of visuospatial but not object identity information for the visual guidance of movement, and, on the other, the importance of object identity but not visuospatial information for object-reward associations.

We have undertaken a collaborative study with members of the Laboratory of Neurosciences of the NIA to investigate the possible presence and location of separate visual pathways in human cortex for processing object identity and spatial location. In this study, regional cerebral blood flow was measured with positron emission tomography (PET) as subjects performed both an object identity and spatial location task. Areas activated more during the object than the spatial task were located in occipitotemporal cortex, whereas areas activated in the spatial but not the object task were located in superior parietal cortex. These results demonstrate the existence in humans, as in monkeys, of two distinct visual processing pathways, although there appear to be cross-species differences in their precise anatomical locations. A correlational analysis between normalized regional blood flow values indicated that the processing of object identity and spatial location both depend primarily on functional interactions between posterior cortical areas in the right hemisphere.

The occipitotemporal pathway and stimulus encoding

Anatomical delineation of the occipitotemporal pathway has opened the way to a study of the functional properties of its single neurons. In an attempt to decode the visual information that they carry, we have developed new methods to identify and quantify the temporal patterns of a neuron's stimulus-evoked discharge. By viewing single neurons in the behaving monkey as communication channels and applying techniques from statistics, signal processing, and information theory to the data, we have found that neurons in both V1 and TE convey stimulus information in several simultaneous, independent, temporal patterns of activity. The amount of information about the stimulus conveyed by these different patterns was nearly twice as great as that transmitted by a more usual measure of the neuronal response, the spike count itself.

Based on these results, we have proposed a new model of the

function of visual system neurons - the multiplex-filter hypothesis - which states that neurons respond to visual stimuli as though they consisted of several, simultaneously active, spatial-to-temporal transforming or filtering mechanisms. The filtering mechanisms are reflected in the independent activity patterns or temporal modulations of the neuronal spike train, which can thus be viewed as containing several messages about the stimulus multiplexed into the spike train. When this hypothesis was tested with a quantitative model, the model accurately predicted the temporally modulated responses of both lateral geniculate and striate cortical complex cells to complex mixtures of basic stimuli. However, using firing rate alone, we have also been able to construct neural network models of striate complex cells (with nonlinear input-output functions) that predict their response to realistic objects, such as shaded three-dimensional surfaces, with a correlation between predicted and actual response of 0.78. Running the network "in reverse" generated new images of what should be the optimal patterns for the cells. Now that we have established some of the basic methodology for studying nonlinear cells, we will apply the techniques to the problems of object recognition in extrastriate areas (see below).

We have examined how closely related the messages are from neurons located close together in primary visual cortex, by recording from pairs of adjacent neurons simultaneously with a single electrode. Informational analysis showed that the messages carried by pairs of adjacent neurons are nearly completely independent, i.e., the response of one neuron cannot be predicted from the response of the other. This independence is highly desirable, for if adjacent neurons, most of whose signals arise from the same sources, were to extract the same information, they would also extract the same random fluctuations, i.e., the same noise. Averaging their signals would therefore not improve the signal, i.e., would not decrease the noise. Based on this analysis of our results, we hypothesize that each cortical module is designed to keep a full representation of the incoming information through distributed encoding.

As part of this research endeavor, we are continuing to develop new ways of assessing the amount of information carried by single neurons and sets of neurons. For example, we recently increased the ease and accuracy of such calculation through use of computational neural networks, and this in turn has improved the accuracy of our quantitative models.

<u>The occipitotemporal pathway and the analysis of color, contour, and shape</u>

Now that we know the location, visuotopic organization, and connections of many of the extrastriate areas in the occipitotemporal pathway, we can trace the transformation of

visual information through them at the single neuron level. We began our physiological analyses in area V4, which plays a crucial role in the relay of visual information into the temporal lobe. We found that, as in striate cortex, some neurons in V4 are sensitive to object contours and low spatial frequencies, whereas others are more sensitive to textures and high spatial frequencies. Thus, the data on V4 suggest that within each visual area of the occipitotemporal pathway, the contours of object surfaces and the textures of object surfaces may be processed by separate populations of neurons. Surface color also appears to be processed by neurons within each of these visual areas. The results argue against the prevalent view that each area of the occipitotemporal pathway processes a different aspect of an object separately, such as color in V4 and depth in V2, and suggest instead that the different features of an object are processed in parallel within each area. At the same time, the results are consistent with our anatomical evidence indicating that the occipitotemporal pathway is organized as a serial hierarchy.

A hierarchical model predicts that the product of visual processing will become progressively more complex at each successive stage in the pathway. So far, our results in V4 support this prediction. In addition to sensitivity to the contour, texture, and color of a stimulus, many V4 neurons respond to a stimulus only if it stands out from its background on the basis of a difference in color or form. This responsivity to stimulus differences in the receptive field is due to a unique receptive-field structure of the neurons in V4: a small excitatory receptive field surrounded by a large, silent, suppressive zone. The surround zone is silent in that stimulation of it alone is without effect, but is is also suppressive in that it has properties antagonistic to those of the excitatory field and, hence, can suppress the response to an excitatory-field stimulus if the surround is stimulated in the same way. Thus, V4 neurons may play a role in separating figure from ground, a fundamental requirement for object perception. Now that we have a better understanding of sensory coding in V4, we will concentrate our future efforts on investigating the mechanism by which coding is controlled by cognitive factors such as attention.

The occipitotemporal pathway and selective attention

Our retinas are constantly bombarded by a welter of shapes, colors, and textures. Since we are aware of only a small amount of this information at any one moment, most of it must be filtered out centrally. Yet, this filtering cannot easily be explained by the known properties of the visual system. At each successive stage along the pathway from the striate cortex into the temporal lobe there is an increase in receptive field size. Many different stimuli will typically fall within these large

receptive fields, and thus, paradoxically, more rather than less information appears to be processed by single neurons at each successive stage. How then does the visual system limit processing of unwanted stimuli? The results of our single-neuron recording experiments in visual cortex of trained monkeys indicate that unwanted information is filtered from the receptive fields of neurons in extrastriate cortex as a result of selective attention.

We trained monkeys to maintain fixation on a target while performing a task that required them to attend selectively to stimuli presented at one visual field location. Irrelevant stimuli were simultaneously presented at a location outside the monkey's focus of attention. When stimuli at both the attended and ignored locations were simultaneously present within the receptive field of a cell in either area V4 or the inferior temporal cortex, we found that the cell responded to stimuli only at the attended location. For example, if the cell was selective for red stimuli, it would respond well if a red stimulus appeared at an attended location but poorly or not at all if a red stimulus appeared at an ignored location, even though it was still in the receptive field. Thus, we have shown for the first time that the processing of unwanted visual stimuli in extrastriate cortex can be blocked as a result of selective attention. Our most recent evidence indicates that attention can also be directed to a specific sensory dimension of a complex stimulus, such as its texture or shape, and that the neuronal processing of irrelevant information may thereby be reduced even further. We propose that it is these extrastriate neural mechanisms for selective attention that enable us to identify and remember the properties of a particular stimulus out of the many that may be acting on the retina at any given moment.

Not only do extrastriate neurons fail to respond to unattended stimuli, but also the magnitude of their response to attended stimuli depends on how much attention, or effort, the animal devotes to the stimuli. Extrastriate neuronal responses to a given stimulus are larger and more tightly tuned when the monkey is discriminating that stimulus from one that is very similar to it than when the monkey is discriminating it from one that is very different. Likewise, behavioral data indicate that the monkeys' discriminative abilities are improved when they are engaged in a difficult task. Thus, when an animal is challenged by a difficult task, it appears to "rise to the occasion" by concentrating its attention, two neural correlates of which appear to be enhanced responses and sharpened selectivity of the neurons that are processing the stimuli used in the task.

To identify the mechanims by which cognitive state modulates cortical activity, we have begun to examine both extrastriate neuronal response and the animal's performance in an attention-demanding task following lesions (or reversible

deactivation) of structures that provide direct or indirect input to area V4 and the inferior temporal cortex. The results so far show that both the lateral pulvinar and superior colliculus play a critical role in the ability to focus attention on a single stimulus and ignore distractors. Whereas chemical deactivation of neither structure affects performance when there is no distracting stimulus in the visual field, deactivation of either has a devastating effect on performance when a distractor is present. This is just the result we would expect if these structures are involved in filtering unwanted information form the receptive fields of extrastriate neurons.

Recently, we recorded from neurons in the superficial layers of the SC while monkeys performed the task that had revealed the attentional effects of colliculus deactivation. We found that colliculus cells give enhanced responses to a stimulus if its location had recently been stimulated by a visual cue that had (automatically) attracted the animal's attention. Likewise, electrical stimulation of focal sites in the colliculus appears to direct the animal's attention to the corresponding site in the visual field. Surprisingly, however, we did not find enhanced responses from colliculus cells when the animal was instructed (cognitively) to attend to the stimulus in the absence of an explicit orienting cue. Thus, cells in the superior colliculus appear to contribute to a circuit for automatically orienting attention to salient peripheral stimuli but not for the cognitive, or "voluntary," control of attention. The results can be accounted for by a new model of attentional control based on a distributed competitive network, and we will be testing this model over the next year.

Whereas dysfunction of the pulvinar or colliculus may impair the ability to focus attention on a single stimulus and ignore distractors, we would expect that dysfunction of the extrastriate visual cortex itself would impair perception, even in the absence of distractors. We now have evidence for just such a role of the extrastriate cortex. In monkeys trained to fixate, we have found that form and color perception are severely impaired in the specific portion of the visual field affected by a partial lesion in extrastriate area V4. By contrast, motion perception is unaffected, which is consistent with our physiological and anatomical results showing that motion information is relayed through areas MT and MST rather than V4. One surprising result of the V4 lesion is that monkeys were unable to match objects presented at different locations in the visual field. Object representations that are invariant over retinal position are crucial to normal visual perception. Our behavioral results suggest that V4 contributes to the construction of such representations, either directly through unsuspected mechanisms in V4 itself, or indirectly through the relay of object information to the inferior temporal cortex.

Although V4 lesions cause severe impairments in recognition, there remains some residual function. Correspondingly, we have now found that many TE neurons can still be activated following V4 lesions, and, thus, there must be an alternate anatomical route into the temporal cortex. Our recent anatomical finding of a direct pathway from V2 to TE via area TEO would explain not only the partial sparing of color and form vision after V4 lesions but also the visual activation of TE neurons after such lesions. We have now begun behavioral studies to test the relative contributions of V4 and TEO to form and color perception.

A visual cortico-limbic circuit and recognition memory

Monkeys that are shown a series of objects once will demonstrate that they recognize them as familiar several minutes later by consistently choosing them over novel objects or by avoiding them in favor of the novel objects, depending on the paradigm (delayed matching or nonmatching-to-sample, respectively). Thus, somewhere in the visual system the single presentation of a series of complex stimuli leaves traces against which a subsequent presentation of those same stimuli can be matched. If they do match, i.e., if the original neural traces are reactivated, there is immediate recognition of familiarity. The area in which the neural traces are first established appears to be the rostral portion of the inferior temporal (IT) cortex, that is, anterior TE, perirhinal cortex, or both, since removals here but not elsewhere in the visual system abolish the animal's ability to recognize objects that it has seen once just a few seconds before. Apparently, this IT cortex contains the traces laid down by previous viewing, and these traces serve as stored representations (in Hebbian terms, cell assemblies) against which incoming stimuli are constantly being compared. In the process, old cell assemblies may either decay or be renewed or even refined, while new ones are added to the store.

In support of this proposal, we recently found evidence of differential responses of single TE neurons in several delayed matching-to-sample paradigms. In one such paradigm, in which information theory was used to calculate the amount of stimulus-dependent information that was carried in the spike trains, we found that all the neurons recorded carried substantially less information about the stimuli presented in the nonmatch condition (0.21 bits) than about the same stimuli in either the sample (0.53 bits) or match (0.54 bits) conditions. Stimulus recognition requires four steps: encoding, storing, comparison of the currently encoded with the stored (i.e., remembered) stimulus, and a match/nonmatch decision. Our results for IT neurons suggest that they could be involved in all these steps, for their activity carries information simultaneously about both the current stimulus and the remembered stimulus.

Most previous electrophysiological studies of memory in rostral IT cortex have been limited to the standard delayed matching-to-sample paradigm, in which a sample stimulus is followed (after a short blank interval) by a test stimulus, and the monkey must indicate whether or not the test stimulus matches the sample. However, a useful neural mechanism for memory must have the capacity to retain information over relatively long intervals that are not "blank" but rather are filled with new stimuli entering the visual system, competing for processing. To study neural mechanisms under these more realistic memory demands, we recorded from IT neurons in monkeys performing a mnemonic task that required them to actively retain items in memory while concurrently viewing new stimuli. We found that the responses of half the cells were suppressed according to how well the current stimulus matched the stimulus actively held in memory. Thus, rostral IT neurons may function as adaptive "mnemonic filters." Temporal contiguity alone could not explain the results, as there was no modulation of responses when a stimulus on one trial was repeated on the next trial; an active reset mechanism appears to restrict the memory comparison to just the stimuli presented within a trial. Responses to stimuli that matched memory traces were suppressed from the earliest onset of the visual response and remained suppressed in a sustained fashion; we found no evidence for significant temporal modulation of the spike trains when we averaged responses over intervening stimuli. To determine how much mnemonic information was conveyed by individual IT cells, we analyzed the responses with discriminant analysis and neural network techniques. According to these analyses, one could achieve the same performance on the task as the animal did by pooling the responses of only 25 IT neurons. Thus, mnemonic information equivalent to that held by the animal as a whole is distributed down to the level of small neural populations.

We propose a model in which working memory is mediated by two populations of IT cells. One functions as adaptive mnemonic filters and the other functions as a sensory referent. The difference in response between the two populations is a measure of how much the current stimulus stands out from stimuli of the recent past, a type of temporal figure-ground extraction.

Our results on working memory were all obtained with stimuli that were highly familiar to the monkey, and the modulation of responses we observed were confined to a single trial of the monkey's mnemonic task (lasting up to about 8 seconds). However, when we began using novel stimuli in the task, i.e. stimuli the monkey had never seen before, we observed longer-term influences on neuronal responses over the course of the hour-long recording session. As novel stimuli gradually became familiar to the animal, we observed a gradual "focusing" or narrowing of activity across the population of neurons. Cells could detect that a particular novel stimulus had been seen before, even after more

than a hundred intervening presentations of other stimuli. Although these changes in the representations of stimuli across the population of cells appeared to be stable for at least an hour, we have just begun to test whether they are retained across days. If so, the changes most likely reflect long-term memory formation. Taken together, our results on working and recognition memory suggest that inferior temporal neurons function as adaptive mnemonic filters operating over a range of time domains.

Significantly, area TE projects directly to the amygdala and indirectly to both the amygdala and hippocampus via perirhinal and entorhinal cortex. In our previous work we had established that at least part of the neural circuit necessary for storing the traces includes the cortico-limbo-thalamo-prefrontal system, which is actually composed of two largely independent systems arranged in parallel. One of these systems consists of the amygdala, amygdalofugal pathways, magnocellular portion of the medial dorsal nucleus (MDmc), and orbital frontal cortex, while the other consists of the hippocampus, fornix, anterior nuclei of the thalamus (Ant N), and cingulate cortex. The evidence that these two systems operate in parallel comes from our finding that damage to the amygdalar and hippocampal systems at any stage (i.e. medial temporal lobe, limbo-thalamic pathways, medial thalamus, or medial prefrontal cortex) causes a severe loss in recognition memory, but only when the two systems are damaged in combination. Damage to just one of the two leads to only mild recognition deficits, suggesting that either system can compensate for loss of the other, at least so far as recognition memory is concerned.

Recent anatomical evidence has indicated that the bed nucleus of the stria terminalis occupies an anatomical position within the amygdalar system that is comparable in some respects to the position occupied by the mamillary bodies within the hippocampal system. That is, just as the hippocampal formation projects to Ant N directly and also indirectly via the mamillary bodies, the amygdala projects to MDmc directly and also indirectly via the bed nucleus of the stria terminalis. These particular relays between the medial temporal lobe and medial thalamus are not normally critical for recognition memory, since combined damage to the two relays yields only a mild impairment. However, they might have an important role in the enhancement of memory by emotion, a possibility that we plan to explore.

Our experimental evidence indicating that combined damage to the amygdalar and hippocampal systems is necessary to produce a disorder in monkeys resembling the syndrome of global amnesia in man is consistent with most of the neuropathological evidence available on amnesic patients, including patients with temporal lobe resections and those with Korsakoff's disease. One piece of clinical evidence does not seem to fit this view, however, and

supports instead the view that damage to the hippocampal formation alone is sufficient to produce the syndrome. The evidence comes from amnesic patients with diseases of the posterior cerebral artery, which is known to provide the blood supply of the hippocampus but not the amygdala. To examine this issue experimentally, we occluded the posterior cerebral artery in the monkey and found a substantial visual recognition loss in several animals, with scores averaging 20% below those of normal controls. These animals had bilateral infarctions confined almost entirely to the hippocampal formation and parahippocampal gyrus, and then only to restricted portions of these structures. Indeed, the only subfields of the hippocampus damaged in common in these cases were CA1 and CA2. Paradoxically, the memory loss found in these animals with only partial bilateral hippocampal damage was significantly greater that that found in animals with total bilateral ablation of the hippocampal formation, whose scores averaged only 10% below those of normal controls. Consideration of the possible mechanisms accounting for this paradoxical finding may be important in understanding the memory deficits found in amnesic patients with partial damage to the hippocampus resulting from hypoxic ischemic episodes as well as from cerebrovascular accidents.

Recently, we have found that a removal limited to the cortical tissue ventrally adjacent to the amygdala and hippocampus, specifically the tissue lining the banks of the rhinal sulcus, results in a severe recognition memory deficit in monkeys. We are currently investigating how this tissue is related to the amygdala and hippocampus, both anatomically and functionally, and whether this tissue makes any independent contributions to memory. So far, we have determined that both the entorhinal and perirhinal cortical components of the rhinal cortex contribute to recognition memory, with the perirhinal cortex making the greater contribution.

The cholinergic system and recognition memory

We have proposed a neural model for recognition memory in which a stimulus leaves a trace or cell assembly in the sensory modality's higher order processing stations whenever that stimulus activates the cortico-limbic pathway described above. But how do the limbic structures bring about that storage, i.e., how do they reactivate and cause synaptyc changes in the cortical sensory neurons that activated them? Based on three major lines of evidence, we suggest that this missing link is the basal forebrain cholinergic system. First, cholinergic agonists and antagonists have long been known to influence many forms of memory in many species, and in our own studies we have found that the same holds true for various forms of memory in the monkey. Moreover, we have found that the muscarinic-receptor blocker scopolamine prevents the storage of information at a very early stage, i.e. between 0 and 3 seconds after stimulus offset,

without affecting perception, i.e. 0 seconds after stimulus offset. Also, scopolamine was found to have no effect if it was administered after stimulus acquisition but before testing, i.e. after storage but before retrieval. These findings suggest that scopolamine blocks primary memory and, thus, that binding of acetylcholine to muscarinic receptors is necessary for any entry of sensory information into a memory store, but not for retrieval from that store. In a related study, we mapped the distributions of nicotinic and muscarinic cholinergic receptors in the adult monkey brain. Both types of receptors are found in all cortical areas, but the muscarinic receptors are more widely distributed across the layers of a given field and have a wider variety of laminar labeling patterns across fields than nicotinic receptors. Nicotinic receptors are found predominantly in the deep part of layer III, where incoming thalamic and corticocortical afferents terminate densely, in keeping with the pattern characterizing the feedforward projections of the sensory processing pathways. Muscarinic receptors, by contrast, are commonly found most densely in the upper and lower layers of the cortex, a pattern that suggests a muscarinic role in the central modulation of sensory processing via the feedback projections of those same pathways. The receptor distribution patterns thus appear to fit the notion that the mnemonic effects of the anticholinergic agent scopolamine are exerted through a blockade of the central modulating system.

The second line of evidence favoring the cholinergic hypothesis involves recent neuropathological studies in patients with Alzheimer's disease, who often show a marked memory loss as one of their earliest symptoms. Postmortem examination of such cases revealed cell loss in both the nucleus basalis of Meynert (nbM), the major source of cholinergic input to the cerebral cortex and amygdala, and the nuclei of the medial septum and diagonal band of Broca (ms/dbB), the major sources of cholinergic input to the hippocampus. This cell loss is accompanied by markedly decreased cortical and limbic levels of choline acetyltransferase and acetylcholinesterase, enzymes involved in the synthesis and metabolism of acetylcholine. In experiments performed in collaboration with investigators from The Johns Hopkins Medical School, we produced recognition memory impairments in monkeys by damaging the basal forebrain with a neurotoxin. The monkeys with the most severe memory impairment had nearly complete destruction of the basal forebrain cholinergic nuclei as well as a 60-90% loss of choline acetyltransferase activity across most of the cortex.

However, there were only small decreases of this critical enzyme in rostral IT cortex, the sector that is so important for visual recognition memory. The results suggest the interesting possibility that the visual recognition impairment was due to cholinergic denervation not of the cortex but of the limbic system. This possibility would also account for the finding that

only combined damage of nbM and ms/dbB yielded impairment, since only such combined damage would result in cholinergic denervation of both the amygdala and hippocampus.

Supporting this interpretation, we now have data from IT neurons recorded in animals that received systemic injections of scopolamine. Although performance of the delayed matching-to-sample task was impaired following 0-3 intervening stimuli, IT neurons continued to show match-nonmatch response differences, just as in the normal animals. Thus, the effect of the systemically administered scopolamine appears to be on structures "downstream" from inferior temporal cortex.

The third line of evidence in support of a cholinergic mechanism comes from a study that was aimed at providing a more complete picture of the anatomical relations between the limbic system and the basal forebrain. In this investigation we found that the amygdala and hippocampus project mainly to the same parts of the basal forebrain that innervate them, namely, the nbM and ms/dbB, respectively, with little overlap between them. Interestingly, because the visual system itself does not project directly to the basal forebrain, a relay through the limbic system would appear to be essential if the visual system is to activate the cholinergic mechanism.

An extremely puzzling aspect of the recognition ability of the animals with basal forebrain lesions is that they recovered fully after several months of testing. Because the cortical markers of cholinergic activity clearly did not recover during the same period, the results raise the possibility that other neurotransmitter systems may have compensated for the loss of the cholinergic system. Results from other research on cortical plasticity suggest a compensatory relationship between the actions of acetylcholine and other neuromodulators, such as norepinephrine and serotonin. Although it is a completely open question as to whether these other neuromodulators are involved in a compensatory process, loss of neurons in the locus coeruleus and raphe nucleus, the major sources of noradrenergic and serotonergic inputs to the cortex, also has been reported in cases of Alzheimer's disease.

In conjunction with these experimental manipulations of the basal forebrain cholinergic system, the goal of which is a better understanding of the nature and neurobiological basis of the cognitive losses associated with Alzheimer's disease, we have begun to examine the cognitive losses associated with normal aging in the monkey. Our initial results demonstrated a moderate but systematic decline in the recognition memory of monkeys from early adulthood (3-6 yrs), through middle age (14-17 yrs), to old age (26-30 yrs), as well as an age-related impairment in the type of spatial memory measured by the classical delayed-response test. Since there was no correlation between the object

recognition and spatial memory deficits in the aged animals, and since the two abilities are known to depend on largely different neural substrates, it is likely that multiple neural systems are vulnerable to the effects of aging and that the vulnerabilities differ from animal to animal. We plan to test this hypothesis directly by histological and histochemical examination of the tissues in question.

Molecular mechanisms of neural plasticity

It is likely that release of acetylcholine into the synapses of neurons in the sensory processing pathways initiates a cascade of cellular neurochemical events that leads to strengthening of the synapses. As a result, many of the neurons whose signals have just represented a sensory stimulus could become linked together in the cell assembly that is posited to be the stored representation of that stimulus. One cellular process that has become a strong candidate in the search for molecular mechanisms underlying neural plasticity is the phosphorylation of a protein band, F1. This protein band was found by investigators at Northwestern University to increase its rate of phosphate incorporation in the hippocampus both after memory formation and after long-term potentiation. In collaboration with these investigators, we have found a protein in the cerebral cortex of the monkey that appears to be homologous to F1 on the basis of molecular weight, isoelectric point, two-dimensional phosphopeptide maps, and phosphorylation by exogenous, purified protein kinase C (PKC). To determine whether regional phosphorylation levels of this protein might be related to information storage, we examined protein phosphorylation along the length of the occipitotemporal visual processing pathway. We found that phosphorylation levels of protein F1 increased in a gradient along the pathway, with levels in rostral inferior temporal and entorhinal cortices approximately ten times those in striate cortex. F1 phosphorylation could thus constitute one mechanism for the cortical storage of visual memories.

Further support for this conclusion has now been obtained in a collaborative study with investigators from NINDS and Johns Hopkins University showing that the amount of PKC was significantly lower within the CA1, CA3, and dentate gyrus subfields of the hippocampus in rats trained to solve a water maze task by cognitive mapping than in control rats trained to solve it by a visual discrimination strategy. Hippocampal lesions interfered with learning only if it was based on cognitive mapping. The results suggest that changes in the distribution of PKC within the mammalian hippocampus play a critical role in memory storage.

A cortico-limbic pathway for tactual object recognition

The work described above elucidating a cortico-limbo-

neuromodulatory system for visual perception and memory has led to the search for analogous systems in other modalities. In fact, analogous anatomical pathways have been tentatively identified in all of the sensory modalities, but, because of our recent finding that combined amygdalo-hippocampal lesions produce severe recognition loss not only in vision but also in somesthesis, particular attention has been paid to the pathway in this modality. Also, anatomical studies on the somatosensory system have been supplemented with electrophysiological and behavioral studies in order to permit functional comparisons with the memory circuit in vision.

Anatomical tracers were injected into the hand representations of physiologically identified somatosensory cortical fields in the postcentral strip and within the lateral sulcus, and the patterns of connections between fields were assessed. Applying principles previously worked out in the visual system regarding direction of information flow based on analysis of laminar projection patterns, and from our own work with combined lesion and recording studies in the somatosensory system, we have started determining the direction of cortical flow of tactual information. Starting from the fields comprising primary somatosensory cortex in the postcentral strip, information is relayed along two pathways, one running dorsally through area 5 and then to area 7, and another coursing ventrally to SII and then onto the insula before reaching the amygdala directly and the hippocampus indirectly via perirhinal cortex. This latter pathway appears to be analogous to the ventrally directed object recognition pathway in the visual system.

In the course of identifying this somatosensory cortico-limbic pathway, we resolved a long-standing difficulty for our serial cortical processing hypothesis, which predicts that the postcentral strip and SII cortex process information in sequence. It was supposed earlier that these two cortical regions were both major projection zones of the ventroposterior nucleus, the primary somatosensory relay of the thalamus, implying that the two cortical areas received and processed information in parallel. Two major findings support our view that SII receives its major source of activation from the cortical fields in postcentral cortex rather from the thalamus. The first is that following injections of tracers in SII cortex, very few labeled cells are found in the ventroposterior nucleus, demonstrating the lack of a major output from this portion of the thalamus to SII. The other finding is that following removal of the somatic fields comprising postcentral cortex, neurons in SII are no longer responsive to somatic stimulation, indicating the lack of a driving input directly from the thalamus. The two findings together demonstrate SII's dependency on the postcentral strip for somatic activation of its neurons and strongly support our suggestion of a sequential ventrally directed corticocortical processing pathway for somesthesis. New physiological and

behavioral investigations are being directed at the insula, since our anatomical data point to this cortex as constituting the next station in a cortico-limbic pathway for tactile recognition. In addition, we have begun neuroanatomical studies to clarify the connectional relations of the somatic areas comprising the dorsal pathway.

Given the above findings regarding a ventrally directed somatosensory pathway, we were interested in determining the contribution of each of the individual cortical areas comprising postcentral cortex (areas 3a, 3b, 1, and 2) to the responsivity and modality properties of SII neurons. We therefore removed one or more of the four postcentral hand representations in various combinations and then examined the effects on the hand representation in SII. We found that removals that left intact only the postcentral areas that process high intensity or "deep" inputs (i.e. areas 3a and 2) yielded mainly SII recording sites responsive to "deep" somatic stimulation exclusively. Conversely, removals that left intact only postcentral areas that process predominantly cutaneous information (i.e. areas 3b and 1) yielded SII recording sites responsive to cutaneous stimulation and none that was driven exclusively by "deep" stimulation. Since the ratio of cutaneous and "deep" sites for body-part representations other than of the hand was not affected by any of the ablations, the results were clearly attributable to the specific lesions. These findings further characterize the nature of processing along the ventrally directed pathway by demonstrating that modality-specific information is relayed from postcentral cortical areas to SII along parallel channels, with cutaneous inputs transmitted via areas 3b and 1 and "deep" inputs via areas 3a and 2.

Lesion-induced cortical plasticity

An unexpected result of the electrophysiological work just described was the finding that the SII region undergoes major functional reorganization after removal of all the postcentral representations of a given body part (i.e., including its maps in 3a, 3b, 1, and 2), though not after a partial removal. Complete ablation of a body-part's maps in the postcentral strip initially silences the map of that same body part in SII, but the affected SII zone does not remain silent; instead, representations of different body parts in the adjacent portions of SII expand to occupy the affected zone. For example, six to eight weeks after total removal of the postcentral representations of the hand, the hand region in SII becomes responsive to stimulation of the foot, which increases its SII representation to occupy most of the former hand region, a linear distance of 5 mm or more. Taken in conjunction with our finding that postcentral cortex is the source of SII's somatic activation, the reorganization of SII provides the first direct evidence in adult mammals that the cortex itself can undergo plastic changes after CNS injury.

Encouraged by this evidence of extensive lesion-induced plasticity in adult primates, we took advantage of an opportunity to examine the neuronal responsivity of the postcentral cortex in a group of monkeys that, 10-12 years earlier, had undergone unilateral or bilateral dorsal rhizotomies of the nerve segments representing the entire upper limb. In a dorsal rhizotomy, sensory nerve roots are severed at a point where they exit the dorsal root ganglion, thereby denervating the brain stem, thalamus, and cortex of this particular input. It was a distinct possibility therefore that the corresponding cortical sensory maps had remained deactivated, i.e. unresponsive to somatic stimulation, since the rhizotomy. We found, instead, that the entire upper limb representation had become reorganized and was now responsive to stimulation of the lower part of the face. This surprising finding extended the previously presumed upper limit for cortical reorganization by an order of magnitude (i.e., from a linear distance of 1 mm to 1 cm, or 1/3 the length of the postcentral strip) and raised the possibility that the upper limit might be even greater.

The mechanisms responsible for these massive reorganizational changes remain a mystery. A clue to one factor, however, has come from a study designed to compare the effects of peripheral sensory lesions with those of the central sensory lesions described above. In this follow-up study, all three nerves normally innervating the hand were cut. Six to eight weeks later, postcentral cortex normally devoted to the hand had become responsive to high intensity somatic stimulation of both the arm and face, but sites in the expected location of the hand representation in SII were still largely unresponsive to any somatic stimulation. We then used immunohistochemical techniques to examine the levels of several different neurotransmitters throughout the somatosensory system of these same monkeys and found a reduction in the levels of glutamate in SII. The pattern of glutamate-immunoreactivity (GLU-ir) in the dorsal horn of the spinal cord, dorsal column nuclei, ventroposterior nucleus of the thalamus, and each of the cytoarchitectonic areas comprising the postcentral strip showed no obvious difference in the animals with nerve cuts from the Glu-ir pattern in normal animals. In SII, by contrast, regions of normal Glu-ir alternated with regions in which Glu-ir was either absent or dramatically reduced. The affected regions were in the hand representation and corresponded to cytochrome-oxidase poor regions seen in adjacent sections. A different result was obtained in two monkeys that had had complete ablation of the hand representations in the postcentral strip. In these animals, which had shown somatotopic reorganization of SII, there was no reduction of Glu-ir or cytochrome oxidase staining in any portion of SII.

Taken together, the electrophysiological and immunohistochemical experiments indicate a close relationship between glutamate

levels and somatotopic reorganization. The evidence is of course only correlative at this point. However, given the well established involvement of the NMDA-type of excitatory amino acid receptor in use-dependent developmental plasticity, our findings suggest that the reduction in glutamate may indeed have been one cause of the failure of cortical reorganization. Future experiments will be directed at testing this possibility.

Amygdala and recency memory

Earlier studies had indicated that removals of the amygdala and underlying cortex produce a deficit on a version of delayed nonmatching-to-sample that employs two repeatedly used objects, a task thought to measure recency memory. Although the impairment was originally attributed to the amygdalar portion of the removal, new studies in which monkeys were given selective neurotoxic lesions of the amygdala or removals of the subjacent rhinal cortical area alone have shown that the rhinal area is contributing to recency memory as well.

Amygdala, hippocampus, and associative memory

According to our neural model, once the trace or representation of a stimulus has been stored in the higher order processing stations of any given modality, that stored trace can enter into association with the stored traces of other stimuli and other events, thereby providing the stimulus with associative meaning. As has been indicated, the amygdala and hippocampus, as well as their separate efferent pathways and separate thalamic targets, make approximately equal contributions to recognition memory, presumably reflecting their roughly equal contribution to the cortical storage of stimulus traces. In the case of associative memory or recall, however, our results indicate that the amygdala and hippocampus make very different contributions.

In one experiment, monkeys were trained preoperatively on a visual recognition task and, separately, on a tactual recognition task, with the same set of objects comprising the stimuli for both modalities. One group of monkeys then received amygdalectomies and the other, hippocampectomies, after which both were retrained on the intramodal memory tasks to a high level of performance. When tested later for their ability to perform the recognition task across modalities, i.e. to choose between two objects visually after one had been presented as a tactile sample, the hippocampectomized monkeys continued to perform at a high level, whereas the performance of the amygdalectomized monkeys fell to chance.

Nearly the opposite results were obtained in a second study that tested the ability of monkeys to remember the spatial location of visual objects. In this task, the animal was required to remember on the test trial where on a three-well tray each of two

different objects had been presented on the acquisition trial. In this case, monkeys given amygdalectomy were able to regain the level of performance they had achieved preoperatively, whereas those given hippocampectomy failed to rise above chance.

The results of these two complementary experiments indicate that, although both the amygdala and hippocampus are critical for certain forms of associative memory, their roles are totally different. Many further analyses along the lines of these experiments will of course be necessary before the selective associative memory functions of the amygdala and hippocampus can be identified with confidence. For example, the association of an object with an affective state, such as fear or pleasure, appears to depend much more heavily on the amygdala than on the hippocampus. New support for this view has been obtained in an experiment showing that one-trial object-reward association is impaired more by amygdalar than by hippocampal lesions, although neither deficit approaches in severity the one produced by the combined removal of these two structures. By contrast, because of the important contribution to spatial memory that is made by the hippocampus, the association of objects with spatially directed motor acts could depend more heavily on the hippocampus than on the amygdala. Studies to examine this possibility are being planned.

In another recently completed study, monkeys were trained on a visual-visual associative memory or recall task. The results suggest that both the amygdala and hippocampus contribute to this kind of memory, but that neither structure is essential by itself. Taken together with the results of the other experiments on associative memory described above, the data suggest that the amygdala may be critical for crossmodal associations, the hippocampus for object-place associations, and that both structures together are essential for intramodal associative memories.

In recent physiological work, we uncovered striking evidence for activation of specific inferior temporal neurons during within-modality recall. The results showed that when an animal is given a brief visual cue (at one spatial location) to search for an item (at a different location), IT neurons coding the searched-for item are activated and remain so until the item is found. Indeed, when a given cue is used predictably for a block of trials, we have seen maintained activation of specific IT neurons for more than fifteen minutes. In area V4, we recently found that when the animal is instructed to hold its attention in a sustained fashion at one location in the visual field, neurons coding that specific spatial location are also activated for long periods in the absence of any stimulus - as long as attention is sustained. The level of sustained activation is often low, only a few spikes per second, but is very reliable.

In addition to the phenomenological experience of "recall," we believe that the function of sustained activation in these areas may be to segregate (or "label") the neurons that will provide the information needed for a given task. This segregation might be facilitated if the higher discharge rate led to synchronization of firing among the selected neurons, a possibility we are now preparing to test.

Nonlimbic structures and habit formation

On all of the memory tasks that have been described, the deficits are especially severe when removals of the amygdala and hippocampus are combined. Yet, even the combined limbic lesion does not affect all forms of learning and retention. For example, despite their rapid forgetting in one-trial object recognition, animals with combined limbic lesions have no difficulty learning object discriminations, at least in the standard situation where trials are repeated 3-4 times per minute. In an attempt to resolve this discrepancy between rapid forgetting and successful learning, we tested whether object discrimination learning would be prevented in animals with limbic lesions if intertrial intervals exceeded the putative memory span. Surprisingly, amnesic animals with the combined amygdalo-hippocampal removals learned to discriminate a long list of object pairs even though the list was presented only once every 24 hours. The same result was obtained in amnesic animals with combined orbital frontal and cingulate lesions, as well as in those with rhinal cortical lesions. Thus, although the operated animals have an extremely short memory span, they can retain and accumulate information gained from single discrimination learning trials separated by 24-hour intertrial intervals.

Soon after discovering this phenomenon in tests with objects, we found that the same dissociation, i.e. impaired recognition but spared discrimination learning despite 24-hour intertrial intervals, holds for pictorial stimuli as well. This paradoxical success in the presence of severe cognitive memory loss implies the existence of a powerful learning and retention mechanism outside the limbic system. To distinguish this form of retention from cognitive memory, we have labelled it 'habit formation', with the implication that it reflects the formation of S-R bonds as a result of the reinforcement contingencies operating at that time.

Because the occipitotemporal pathway is known from behavioral evidence to form the initial part of the system underlying visual habit formation, and because other behavioral evidence suggests that the basal ganglia could also play a role, we have begun to explore the projections from the occipitotemporal pathway to the neostriatum. So far, we have found that areas TE, TEO, and V4 all project to the tail of the caudate nucleus and to the ventral

portion of the putamen. This arrangement contrasts with the pattern of projections from the occipitotemporal pathway to the limbic system, which arise from TE only. The presence of direct projections to the striatum but not to the limbic system from areas V4 and TEO may explain the ability of monkeys with TE lesions to acquire visual habits after sufficient training but not cognitive visual memories. We also found that the efferent projections of both the tail of the caudate nucleus and ventral putamen are confined to the globus pallidus (GP) and substantia nigra, pars reticulata (SNr). Because the GP and SNr are known to project via the thalamus to the dorsolateral premotor and supplementary motor cortex, respectively, these cortical regions may represent further stations in the neural circuit of the postulated habit formation system. By delineating the wiring diagram of this system, we have identified structures to be targeted for interventional neurobehavioral studies.

In our first such study, we trained animals on an initial set of object discriminations with 24-hour ITI's, and then injected the neurotoxin ibotenic acid bilaterally into the tail of the caudate nucleus and the adjoining ventral putamen using stereotactic coordinates derived from magnetic resonance imaging, which was also used to verify the accuracy of the lesions postoperatively. Subsequently, all animals required more than twice the number of trials to learn the discriminations with two new sets of objects. When tested on a visual recognition test, however, the animals performed as well as normal control animals. This pattern of impairment strongly suggests that visual recognition memory and visual habit formation are mediated by separate pathways and that the tail of the caudate nucleus and the ventral putamen are important components in the pathway for the latter type of learning.

Against the idea that the tail of the caudate nucleus participates in habit formation is earlier evidence that neurons in this structure respond very poorly to visual stimuli and habituate rapidly, even during performance of a visual discrimination task. To reexamine this issue, we have begun to record from the tail of the caudate nucleus and ventral putamen while monkeys performed previously learned visual discriminations (in a go/no-go paradigm) and also while they learned new ones. Unlike the earlier evidence, we found that the neostriatal cells were both highly responsive and highly stimulus-selective, resembling cells in cortical areas such as area TE that project to the neostriatum. The most interesting data, however, are from a subpopulation of neostriatal cells that change their response during learning. As the animal learns, these cells appear to become more responsive to the no-go stimuli, i.e., the stimuli that do not lead to reward and thus do not elicit a behavioral response. Clearly, we have just scratched the surface on the role of neostriatal neurons in learning and will be pursuing this over the next year.

Another behavioral paradigm that may provide a measure of the ability to acquire habits is mastery of the principle of delayed nonmatching-to-sample (DNMS). Although combined amygdalo-hippocampal removals in macaques severely impair their performance on DNMS when delays between sample and choice exceed about 10 seconds, they can master the task with shorter delays. Such mastery cannot depend on the formation of specific visual discrimination habits, because (a) a different pair of objects is used on every trial and (b) within a trial, the reinforcement contingencies for responses to the sample object are inconsistent. To master the task in the absence of the limbic system, the animal must be able to learn a principle or rule, which requires, in turn, (i) suppression of specific stimulus-response habits, (ii) abstraction of sameness and difference from specific stimulus quality with the aid of immediate memory, and (iii) formation of a stimulus/ difference-response habit. We have now found that if inferior prefrontal lesions (which produce a only a mild DNMS impairment by themselves) are added to amygdalo-hippocampal lesions, monkeys lose the ability to perform DNMS even when the delays are less than 10 seconds. This finding suggests that the inferior prefrontal cortex serves one or more of the processes described above needed for rule learning.

To determine which of these various processes might be most dependent on the inferior prefrontal convexity, we evaluated the effects of combined inferior prefrontal and amygdalo- hippocampal ablations on two additional tasks designed to tease the processes apart. The first task, delayed matching- to-sample (DMS), is almost identical to DNMS except that it does not require the suppression of specific stimulus-response habits (see i, above). Preliminary results indicate that monkeys with the combined prefrontal and limbic lesions fail to relearn DMS just as they failed to relearn DNMS. Thus, the critical contribution of the inferior prefrontal cortex to performance on DNMS cannot be linked solely to the process of suppression of specific stimulus-response habits.

In the second task, we removed the requirement for immediate memory but retained the requirement for same/different discrimination (see ii, above). This was achieved by training the monkeys to discriminate like from unlike pairs of trial-unique objects presented simultaneously, with the like pair being designated correct. In this experiment, each animal with the combined prefrontal and limbic lesions was able to relearn the task to a high level of accuracy, suggesting that neither inferior prefrontal cortex nor amygdalo-hippocampus, singly or in combination, are essential for making same/different judgements. Taken together with the failure of animals with the combined leasions to learn either DNMS or DMS, the evidence suggests that both the inferior prefrontal cortex and the amygdala-hippocampus can mediate primary visual memory singly, but in the absence of

both, primary visual memory is abolished.

Ontogenetic development and decline of cognitive memory and habit formation in infant and aged monkeys

Our evidence from studies in adult monkeys suggests that cognitive memory and habit formation are two qualitatively different retention processes based on separate neural mechanisms. Our studies of behavioral development in infant monkeys provide complementary evidence by suggesting that these two systems are developmentally dissociable, in that the limbic memory system appears to mature considerably later than the nonlimbic habit system. A similar delay in the maturation of the limbic memory system has been demonstrated recently in human infants. Our goal is to pursue studies in both monkeys and humans to determine how recognition memory measured by preferential looking differs from recognition memory measured by problem solving. This will help determine which capacities of the memory system appear late in ontogenetic development and, by implication, whether the phenomenon of infantile amnesia might be due to the absence of a fully functional cognitive memory system in early childhood.

To see how cognitive memories and habits develop in animals with early brain damage, we have prepared monkeys with neonatal removal either of the limbic system (i.e. combined amygdalo-hippocampal removals) or of area TE. With regard to habit formation, the results so far indicate that, whereas female infant monkeys can form a set of visual discrimination habits almost as quickly as adults, male infants are significantly retarded. In addition, whereas limbic lesions in both infants and adults leave habit formation intact, neonatal ablation of area TE impairs the learning of female but not of male infants, even though monkeys of both sexes are impaired when the lesions are made in adulthood. These data suggest that ontogenetic development of the habit system is sexually dimorphic, this system maturing earlier in females than in males, presumably because, at this age, area TE or some connected region is more fully developed in females than in males. This sexual dimorphism seems to be dependent on the high testosterone levels found in male infants before and shortly after birth, because a significant correlation appeared between their testosterone levels and learning scores (the more testosterone the poorer the performance), and also because orchiectomy in male infants speeds their rate of habit formation whereas dihydrotestosterone in ovariectomized females retards their rate of habit formation.

With regard to formation of cognitive memories as measured by visual delayed nonmatching-to-sample, the infants with limbic lesions are severely impaired at 10 months and 2 years of age, whereas those with TE lesions show significant and permanent functional sparing at both ages (compared to adults given TE

lesions). The results thus point to a greater compensatory potential after neonatal cortical than after neonatal limbic removals and are consistent with the notion that association areas of the cortex are less mature at birth, and may thus possess greater plasticity, than limbic structures. Direct evidence of neocortical immaturity in the macaque has been provided by our recent behavioral studies showing that cortical areas (such as PG, TF, TEO, and STP) not normally involved in object recognition in adulthood, may assume a critical role in this memory function when area TE has been removed in early infancy; by our anatomical studies indicating the presence at birth of transient connections between area TEO and limbic structures that persist following early damage to area TE, providing a specific compensatory mechanism that could operate to promote recovery of visual memory function following early damage to area TE; and by our neurochemical studies showing that (a) the distribution of both opiatergic and cholinergic receptors is adult-like at birth in subcortical structures and allocortical areas but is not yet fully developed in neocortical areas, particularly the association cortex, and (b) adult levels of metabolic activity in visual association cortex and particularly area TE are not reached until about 6 months of age. These behavioral, anatomical, and neurochemical findings suggest that the relatively poor recognition ability of normal neonates is due more to slow maturation of the cortical association areas than to neonatal immaturity of the limbic system.

We have also investigated the socio-emotional behavior of the infants with neonatal lesions. Early damage to limbic structures does not yield the Kluver-Buchy symptoms of loss of fear, indiscriminate approach to objects, often orally, and coprophagy, seen in monkeys with limbic lesions done in adulthood. Nonetheless, monkeys with neonatal limbic lesions show numerous socio-emotional abnormalities as they mature, such as a lack of social interaction and motor sterotypies. These behavioral abnormalities began to appear when the animals were 6 months of age and persisted when the animals reached adulthood (6-7 years), indicating that early damage to the limbic system yields long-lasting socio-emotional disturbances. In addition, the nature and developmental time-course of these disturbances resemble those seen in autistic children. This finding, together with the recent report by Kemper et al of neuropathology in the amygdala, hippocampus, and cerebellum in each of five autistic subjects, provide evidence that early dysfunction of the limbic system may be one cause of infantile autism. In addition they indicate that the amygdala and the hippocampus are components not only of a limbo-thalamic system serving cognitive functions but also of a limbo-hypothalamic system serving emotional functions. Though much more testing over a much longer time course is necessary, it is becoming clear that the same neonatal damage that leads to a severe cognitive memory disorder can also have extremely serious consequences for personality and social

development, in part because the cognitive memory disorder is present from infancy onward, but also because of the direct effect of the limbic lesions on mechanisms of emotionality. Interestingly, the animals that had received early damage to area TE showed none of the disturbances seen in animals with limbic damage, but they did display other behavioral abnormalities such as hyperactivity and increased frequency in shifting behavioral activities, behaviors that resemble those of children with an attention-deficit hyperactivity disorder.

Our studies in normal aged monkeys, conducted in collaboration with investigators from The Johns Hopkins University School of Medicine, indicate a gradual decline in learning and memory abilities with normal aging. This decline was apparent in spatial abilities as early as the late teens but did not affect cognitive memory or habit formation until the late 20s, indicating that the cerebral systems underlying spatial abilities are compromised earlier than others. At the same time, the behavioral impairments within a given task vary greatly from one aged animal to another, suggesting that different animals have different patterns of cerebral involvement. This possibility is currently being investigated directly through postmortem localization of neuritic plaques and depletion of cholinergic and other neurotransmitters.

Summary

Through combined use of behavioral and neurobiological methods, we are beginning to discover some of the general principles along which the primate forebrain is organized to serve perception, attention, recognition, and recall, as well as some noncognitive learning processes.

Perception. Each primary projection area in the cortex seems to be the source of two multisynaptic corticocortical pathways. Both pathways are composed of several cortical areas that are arranged hierachically, one pathway being directed dorsally to the frontal motor system, the other ventrally to the temporal lobe components of the limbic system. The dorsal pathways from the several modalities are critical for spatial perception and motor guidance. The ventral pathways, by contrast, are critical instead for the perception of objects or stimulus quality and, ultimately, for triggering the motor response.

Attention. In successive stations of each sensory pathway, single neurons carry messages from progressively wider sensory fields but about progressively more specific stimulus configurations. These messages are conveyed not simply by discharge frequency but by means of a temporal code, which appears to contain several simultaneous but independent messages about a stimulus, such as its form, intensity, and duration. Selective attention and attentional effort, reflecting central

influences on sensory processing, can markedly alter the stimulus messages sent by individual sensory neurons, effectively reducing their receptive fields or narrowing their stimulus filters, or both. These selective attentional effects are mediated in part by interaction between the cortical sensory processing systems and subcortical structures including the pulvinar and superior colliculus.

Recognition. Stimulus recognition depends not only on attentionally filtered stimulus processing along the ventral cortical pathway but also on storage of a central representation of that stimulus (in Hebbian terms, formation of a cell assembly) largely, though not exclusively, in the ventral pathway's last station, located in the anterior temporo-insular region. This region projects to the amygdala directly and, via rhinal cortex, indirectly to both the amygdala and hippocampus, and these three limbic structures project in turn to the medial and midline thalamus, with further connections to ventromedial prefrontal cortex. All three of these cerebral regions (limbic, thalamic, and prefrontal) project to the basal forebrain cholinergic system and indirectly to other modulatory neurochemical systems, which innervate the entire cortical mantle. It is hypothesized that a cell assembly representing a configurational stimulus is formed only if this cortico-limbo-neuromodulatory-cortical circuit is activated. The resulting transmitter release may trigger protein phosphorylation and gene expression, to yield increased synaptic efficacy among some of the cortical neurons in the ensemble that just participated in conveying a stimulus message, and thereby form a stable cell assembly representing that stimulus. The slow ontogenetic development of cognitive memory ability, and, by implication, the phenomenon of global amnesia for the experiences of infancy, are probably the result of a slow development of the mechanisms that are needed for the formation of stable cell assemblies (and, as described below, stable phase sequences).

Recall. Once a cell assembly has been formed, it can enter into association with another cell assembly (forming, in Hebbian terms, a phase sequence), thereby providing a stimulus with meaning. Cell assemblies appear to be linked in phase sequences not directly, however, but only indirectly via limbic structures, at least initially. Thus, crossmodal stimulus-stimulus associations, as in recalling how an object looks by feeling it, appear to depend on cortico-amygdalo-cortical interactions, while stimulus-affect associations, as in fear, probably depend largely on cortico-amygdalo-hypothalamic interactions. By contrast, stimulus-place associations, as in recalling where objects are located, seem to depend on cortico-hippocampo-cortical interaction; in this case the hippocampus may act as the link between the ventral stimulus-recognition pathway and the dorsal spatial-perception pathway. Stimulus-act associations could depend on interaction, via the limbic system, between the ventral and dorsal pathways within the lateral prefrontal cortex, which

interacts in turn with the frontal motor system. Finally, stimulus-stimulus associations within a modality also depend on limbic mediation, but in this case they can be mediated by either the amygdala or hippocampus.

Habit formation. Destruction or disconnection of the limbic memory system does not affect all forms of learning and retention. At least one noncognitive form, which has been labeled habit formation, remains intact, presumably reflecting the operation of a powerful cortico-nonlimbic system for learning and retention. This system mediates the acquisition of specific stimulus-response connections, and it appears to do so through sensory-neostriatal interaction.

Rule learning. Mastery of such tasks as delayed matching or nonmatching-to-sample requires learning a rule, which can be accomplished either cognitively or noncognitively. If it is achieved cognitively, i.e. by first abstracting the two categories 'familiar' and 'novel' from the physical qualities of the stimuli and then associating one of these categories with reward, the rule learning is mediated by interaction between the ventral cortical sensory processing pathways and the limbic system. However, the rule can also be acquired as a high-level noncognitive habit, i.e. in the absence of the limbic system, by first abstracting the two categories 'same' and 'different' from stimulus quality with the aid of immediate or short-term memory and then learning through differential reinforcement to respond to one of these categories and to avoid the other. In this case the rule learning appears to be mediated by interactions between the ventral cortical sensory processing pathway, the inferior prefrontal cortex, and the rostroventral neostriatum. In the absence of both the limbic and prefrontal targets of the sensory processing pathways, the ability to learn such rules is abolished, though only if short-term memory is required. Apparently, short-term memory of a stimulus, presumably in the form of a dynamic trace or reverberatory circuit, depends on reciprocal interaction between the ventral cortical sensory processing pathway and either one of two of its major targets - the limbic system or the inferior prefrontal cortex.

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Annual Report of the
Laboratory of Psychology and Psychopathology
National Institute of Mental Health

October 1, 1991 to September 30, 1992

Allan F. Mirsky, Ph.D., Chief

This report summarizes the twelfth year of activity of the program of the Laboratory of Psychology and Psychopathology (LPP) under the direction of Allan F. Mirsky. The full-time professional staff now consists of, Drs. Mirsky, Seymour S. Kety and Theodore P. Zahn (senior staff) Senior Staff Fellow Loring J. Ingraham, Staff Fellows Daisy Pascualvaca and Mary Kosmidis, and Fogarty International Fellow, Miriam Levav. Drs. Pascualvaca, Kosmidis and Levav were all appointed to LPP during the past year .

The LPP staff includes Dr. John Ingeholm, a biomedical engineer, who was recruited to the LPP in 1988 and Dr. Barbara P. Jones, a neuropsychologist, who has been with us on a part-time basis since 1986. Although Dr. Jones intended to retire from an active role in the laboratory this year, she has been able to remain associated with LPP on a part-time basis. It is our hope that this will continue indefinitely. Guest researchers include Drs. Connie C. Duncan, Bryan Fantie, Frances H. Gabbay, Thomas Robinson, Michael Sherer and Lee Mann.

Progress in the various projects continues to be satisfactory and a number of important new findings have emerged which have been detailed below. Two new projects have been undertaken during the past year; these are described at the end of this section.

Our major extramural collaborations are proceeding well. Among those of long-standing are joint projects with the Prevention Intervention Research Center of the School of Hygiene and Public Health of Johns Hopkins University and the Oranim (Institute for Kibbutz Education) of the University of Haifa (Israel). We have recently completed a third assessment of attentional functions in a group of school children in East Baltimore. The project in Israel has now been completed with a twenty-five year followup of the original group of high-risk subjects and controls, and rediagnosis of many of the parents of the high-risk subjects. Other major joint efforts are with Dr. Kenneth Kendler of the Medical College of Virginia. This involves studies related to the genetics of neuropsychological deficit in schizophrenia and schizotypy. The research is being conducted in Ireland and in Virginia. Our collaboration with Dr. Ann Streissguth of the University of Washington continues. This joint effort with the Pregnancy and Health Study of the University of Washington in Seattle involves an assessment and long-term followup of attentional behavior in children (now age 14) whose mothers' drinking records were documented during pregnancy. These were "social" drinkers. Scores on attention tests (the CPT) continue to correlate highly with maternal drinking during pregnancy: the more alcohol consumed, the poorer the attention in the offspring.

Two new collaborations have been undertaken: one is a joint venture with the Department of Psychiatry at Johns Hopkins University (Dr. Ann Pulver) involving neuropsychological assessment of a large group of schizophrenics and their first-degree relatives. The intent is to develop behavioral markers that differentiate among groups of schizophrenic patients (e.g., early vs. late onset, or winter-born vs. other). The presumption is that there may be different

etiologies in those subgroups, which differences may help guide the genetic analysis and typing of this cohort of subjects.

The other new collaboration is with investigators in the Neuroscience Institute (Dr. Marcelo Cruz) of Ecuador. A group of epileptic children and their family members will be assessed with respect to attentional skills. These data will be correlated with the results of analysis of CT scans and EEG examinations. This project is being conducted with an evaluation of the effects of anti-helminthic medications on cognitive functions in these children.

Data from these collaborations have been incorporated in this summary and in the relevant individual project reports.

A brief outline of studies appears below.

A. Clinical Studies of Attention and Brain Function

1. Human Clinical Studies of Attention Disorder
2. Attention Disorders as Assessed by Event-Related Brain Potentials
3. Neuropsychological Evaluation of Psychiatric and Neurological Patients

B. Autonomic Nervous System Activity in Attention and Psychopathology

1. Psychophysiological Responsivity and Behavior in Schizophrenia
2. Psychophysiological Concomitants of Minimal Brain Dysfunction in Children
3. Personality Factors and Psychophysiological Responses to Changing Stimulus Input

C. Interaction of Nature and Nurture in Disordered Behavior

1. Studies of Heredity and Environment in Schizophrenia
2. Studies on Etiological Factors in Schizophrenia
3. Genetic Factors in Response to Alcohol

A. Clinical Studies of Attention and Brain Function

1. Human Clinical Studies of Attention Disorder

We have been involved in a joint enterprise in which a variety of cognitive functions (with special emphasis on attention) are applied to a number of populations of experimental and control subjects. In some instances, autonomic and electroencephalographically-derived tests are administered to the same subjects. The populations have included epileptic persons, schizophrenic subjects, brain-lesioned subjects, dementing subjects, women with eating disorders, dyslexic men, Job Corps Trainees (including a sub-group which is HIV-positive), first degree relatives of schizophrenic and epileptic patients and controls. The aim is to develop a profile of functioning for the several groups that will highlight the similarities and differences among them, will lead to a better neuropsychological characterization of their impairment and provide insights into the pathophysiology of the non-lesion groups. In addition, attention measures are being applied to groups of normal and disordered pre-school and grade school children so as to be able to assess the prognostic value of early developmental signs of

attention disturbances.

The major elements within this program include: the experimental paradigms of the Cognitive Psychophysiology Unit (Dr. Connie C. Duncan, head, guest worker, Dr. Bruno J. Anthony, guest researcher, Dr. Frances H. Gabbay, guest researcher); the Neuropsychological Test Battery supervised by Dr. Jones (consultant) and Dr. Duncan; and the Autonomic Nervous System studies of Dr. Zahn.

The bulk of the neuropsychological examinations were conducted by Ms. Marie Elliott (now retired), with the participation of Drs. Jones and Mirsky and of several psychologists who are employed on a contract basis. Dr. Bryan Fantie, a guest researcher from American University, with the involvement of graduate students in masters and doctoral programs, also participates extensively in the testing of normal control subjects, neuropsychiatric patients and their first-degree relatives. Dr. Duncan's Unit is applying techniques of cognitive psychophysiology to elicit and evaluate event-related potential components (auditory and visual) which have been shown to relate to attention, uncertainty and surprise (P300, N140, etc.). Similar experimental paradigms (e.g., the "oddball" method) have been applied to various groups of subjects with attentional disorders (eating disorders, schizophrenia, dyslexia, etc.) so as to be able to compare and contrast ERP component amplitudes, latencies and distributions. It is anticipated that these studies will help to illuminate (in conjunction with other methods) the nature of the similarities and differences among groups of neuropsychiatric patients whose symptoms include impaired attention. Dr. Zahn's work is reported below.

2. Attention Disorders as Assessed by Event-Related Brain Potential

New Event-Related Brain Potential (ERP) findings have emerged during past two years which contribute to the characterization of cognitive/attentive disturbances in patients with various types of neuropsychiatric disorders. In the Annual Report for 1990-1991, we highlighted the findings on patients with schizophrenia and seasonal affective disorders. It was found that schizophrenic patients show major differences in the visual but not the auditory P300 component of the ERP as a function of successful drug treatment. These data emphasized the possible roles of visual P300 as a state marker and auditory P300 as a trait marker in schizophrenia. A preliminary report of these findings has now been published and a more complete manuscript is in preparation.

In conjunction with investigators from the Neuropsychiatry Branch (Drs. Egan and Wyatt), Dr. Duncan has shown correlations between certain event-related potential measurements and the sizes of structures in the temporal lobe and elsewhere (MRI scans) of patients with schizophrenia. This work may help to explain the sources of the reduced P300 waves in these patients. This work is currently under editorial review.

Dr. Duncan has submitted a report for publication (which is currently under review) describing ERP findings in seasonal affective disorders (SAD). Patients with SAD process visual (but not auditory) information more effectively if they respond to phototherapy. In a small number of patients studied intensively, it has been found that the visual P300 changes are incremental and can be monitored on a day-by-day basis.

Other studies include the following: ERP (P300) Evaluation of sequential probabilities in schizophrenia (under editorial review); P300 in eating disorders; P300 in adult dyslexia; P300 in closed head injuries. The latter three are in preparation. More details are found in the project description of Z01 MH 00509-05 LPP.

A report has been published describing the results of an ERP study of visual and auditory information processing in patients with absence epilepsy. The absence patients displayed markedly reduced P300 amplitudes to stimuli in both visual and auditory tasks. However, in one difficult auditory processing task (an auditory--tone discrimination--version of the AX form of the Continuous Performance Test or CPT), there were virtually no P300s elicited in the patients. Recent studies of patients with complex partial epilepsy show that the lack of P300 to complex auditory stimuli does not occur in that group but is confined to those with an absence seizure disorder. This ERP difference was mirrored by major impairment in the absence patients' ability to execute correct responses in the auditory CPT--in both X and AX forms of the test. Thus, the normal subjects were able to execute both visual and auditory CPT task at the level of 90% correct or higher; in contrast, the performance of the patients with absence epilepsy was quite variable, ranging from 90% correct on the X visual task to 65% correct on the AX auditory task. These data on absence epilepsy suggest a similarity to the findings with auditory information processing seen in schizophrenic patients described above: In both groups of subjects, there may be some overlap in the underlying pathophysiology which renders the processing of auditory stimuli more difficult than visual.

3. Neuropsychological Evaluation of Psychiatric and Neurological Patients

Our battery of neuropsychological tests provides a complete assessment of the executive, mnemonic, linguistic and attentive capacities of the human brain. The test battery has been administered in whole or in part to several hundred persons so far, and the information it provides will form the neurobehavioral database which will be used to interrelate, evaluate and integrate the various electrographic, biochemical and other physiological measures applied to the groups of patients with psychiatric and neurological disorders we study in the LPP. Two fruits of this neuropsychological effort have been published, and at least two more are either under editorial review or in press at present. One publication addresses an analysis of the elements of attention, based upon factor analytic techniques applied to scores from our battery of attention tests. This analysis suggests that separate aspects of attention are assessed by different neuropsychological tests and can in turn be related to separate regions of the central nervous system. This "elements of attention" model appears to have considerable utility in the study of neuropsychiatric disorders and may be of heuristic value in thinking about the process of attention, as well.

The other work (published in the *Journal of Clinical and Experimental Neuropsychology*) concerns a neuropsychological profile of women with various types of eating disorders. There are a number of distinctive profiles associated with different subtypes; e.g., restrictors appear very different neuropsychologically from normal-weight bulimics. Further, many of the cognitive differences from controls appear to be related to a core impairment in attention. Additional details can be found in the project description of Z0I MH 00509-05 LPP.

Parts of the attention battery have been administered to over 500 school children from

the Baltimore Public School System in conjunction with the Prevention Research Center efforts. Two follow-up assessments of nearly 300 of these children have been conducted, and the results are being analyzed at the present time. This is a unique data base, in that the same cohort of children whose shy and aggressive behaviors are also being monitored, is being followed to determine the outcomes of early-identified dispositional (aggressiveness/shyness) and cognitive (attentional elements) behaviors. The results of the analyses of these data may provide a new way of conceptualizing attention and concentration deficits in school children; this, coupled with the experimental interventions that have been implemented, may help in the development of new remedial methods.

Dr. Miriam Levav administered the LPP Attention Battery to a group of children with absence seizures and their first-degree relatives (siblings, parents). She found that the poorest scores were obtained by the epileptic probands, and the next lowest scores by the siblings.

Although the parents are seizure free, the results indicate that the mothers have lower scores than the fathers, mirroring the poorer performance of the female (as compared with the male) probands. These and other findings suggest that the absence seizure disorder may be inherited in the female line, which is consistent with the results of genetic studies of non-focal epilepsy.

B. Autonomic Nervous System Activity in Attention and Psychopathology

This work is being carried out by Dr. Theodore Zahn.

The central focus of this research is the role of attentional processes and autonomic nervous system (ANS) functioning in psychopathology, especially schizophrenia. Studies are directed toward several basic issues: (1) the nature of the attention and ANS dysfunction, (2) the diagnostic specificity of the dysfunction, (3) state vs. trait issues, (4) the neurobiological basis of attention and ANS functioning.

We are continuing to collect data on our current schizophrenia protocol in collaboration with Dr. Pickar of NSB. We study ANS activity during rest and task performance, compare ANS responses to stimuli differing in signal value with respect to frequency, amplitude, and habituation, and determine the nature of attention deficits in schizophrenia using several reaction time (RT) protocols. We test patients when drug-free in most cases, on standard neuroleptic medication, and on more experimental treatments. These currently include the atypical neuroleptic clozapine and idazoxin, a noradrenergic alpha-2 receptor antagonist. The information on how variations in pharmacology affect ANS measures may give us clues as to how neuroleptics alleviate symptoms. In addition, since much of the recent literature on ANS activity in schizophrenia is on only medicated patients our studies should provide information as to the extent to which those results are influenced by medication. A comprehensive review of the ANS psychophysiology of schizophrenia was published last year in the Handbook of Schizophrenia.

We now have completed data analyses from 25 subjects, all of whom were tested on clozapine and either placebo or a standard neuroleptic. Twenty were tested on all three

treatments. On the ANS measures, compared to both other treatments clozapine markedly reduced all aspects of electrodermal activity, increased heart rate, and decreased heart rate variability probably largely due to its anticholinergic and antihistaminic properties. However, vasomotor responses, which are thought not to involve cholinergic synapses, were similarly attenuated, suggesting that clozapine may have central actions that reduce ANS activity. Attempts to predict which patients would improve on clozapine compared to the other two treatments were not markedly successful. The best predictor was that patients showing a good response to clozapine had a smaller increase in tonic ANS activity to a task when on the alternate treatments than did clinical nonresponders. Although clozapine attenuated this tonic ANS response compared to the other treatments, the good clinical responders to clozapine were less affected than the nonresponders. In general, the data suggest that a good clinical response to clozapine compared to a standard neuroleptic is accompanied by a selective focus of relatively greater ANS activity elicited by more important stimuli and/or situations and less by unimportant ones. We are continuing to collect data with this paradigm in order to attempt to replicate these results.

Two reaction-time (RT) paradigms to assess attentional changes have been done on these subjects. One measures simple RT and the other assesses sensory dominance by measuring RTs to lights and tones in simple and choice RT procedures, and on 'conflict' trials in which they were presented simultaneously. The latter paradigm was introduced in part to test the hypothesis that schizophrenics who had auditory hallucinations would show an atypical bias to attend to auditory rather than visual stimuli. Eight of the 25 patients were too psychotic to perform the RT tasks on placebo. However, among the remaining patients, there was no evidence of improvement in RT by clozapine on the average in either paradigm compared to placebo, and RTs under the standard neuroleptic were nonsignificantly better than those on clozapine. On the sensory dominance paradigm patients, like normal controls, were vision-dominant as evidenced by faster RTs to lights than to tones on choice and conflict trials. These effects were significantly reduced under clozapine treatment. On conflict trials, subjects on placebo and neuroleptics showed more failures to respond within 2000 msec to the tone than to the light. This difference was significantly reduced on clozapine. In medicated patients non-responses to the tone were more frequent in those with hallucinations. The results suggest that clozapine may increase the ability of schizophrenics to process nondominant or unattended stimuli possibly by increasing the efficiency of resource allocation and that this may be partially mediated by a reduction in hallucinations. The data also suggest that subjects with hallucinations rather than being audition dominant as hypothesized may have a bias to disattend to external auditory stimuli. Two papers on the clozapine study are in preparation.

We are studying ANS activity and attention in childhood onset schizophrenia in collaboration with Dr. Gordon of CHP. The children are studied on placebo, haloperidol, and clozapine. Results for the first 5 children studied under placebo show that compared to controls they showed excessive spontaneous ANS activity but lower responsivity to novel and meaningful stimuli similar to our previous findings with adult schizophrenics.

A project with CHP on 34 boys with diagnoses in the spectrum of Disruptive Behavior Disorders (DBD) -- Conduct Disorder (CD), Oppositional Defiant Disorder, and/or Attention-Deficit Hyperactivity Disorder (ADHD) -- has the general objectives of determining the nature of the attentional dysfunction and biological mechanisms contributing to these disorders, and if

the objective data for the subgroups support the concept that they form a spectrum. The results from both the attention and autonomic measures, which have been described in detail in previous annual reports, do support the spectrum concept. However, there are differences in ANS baselines and aggression between boys with and without a subdiagnosis of CD which, based on previous studies, suggest that those with low baseline ANS activity would be expected to be more at risk for future antisocial behavior. A paper on the attention measures from this project has been published, and one from the ANS measures has been submitted for publication. We are currently studying a younger sample of ADHD boys with similar methods in order to attempt to replicate the previous results.

Dr. Kruesi of CHP has completed a clinical 2-year followup on 29 of the DBD subjects. We have examined 4 ANS variables as predictors of several outcome variables. In general, the data indicate that low baseline EDA and/or HR are predictive of poor outcome, but the only significant age-corrected relationship is between skin conductance level and institutionalization. The subjects are still not at maximum risk for antisocial behavior, so future followup studies figure to be more definitive.

We have a number of interesting findings on the relationships between biogenic amines from CSF and ANS activity which were described in last years annual report. We are planning to analyze a similar set of data from a group of children and adolescents with obsessive compulsive disorder (OCD) as a comparison group before deciding how to publish these studies. Similarly, we hope to extend our study of ANS activity and personality factors in normal children, described in last years annual report, by including additional normal subjects and looking at similar relationships in the OCD group. We are also planning to compare ANS data in the OCD group with that of normal controls and DBD boys.

In ongoing studies relevant to the neurobiological mechanisms underlying our ANS measures, we have tested the patients with focal head injuries, who were recruited by LPP, on the ANS-attention battery we use with schizophrenics. This should help us determine if the deficits we see in schizophrenics could be due to localized brain dysfunction. We have begun a new study in collaboration with Dr. Grafman of NINDS on subjects with focal brain lesions, with special interest in the frontal lobe. We will use our standard ANS protocol and also test ANS responses to pictures with emotional content in order to attempt to replicate and extend a finding in the literature that some frontal patients are remarkably unresponsive to such pictures.

We have developed methods to analyze respiratory sinus arrythmia which is purported to be a good index of parasympathetic (vagal, cholinergic) control of heart rate. Using data from normal children and adolescents we have found that various measures which have been proposed are highly correlated, highly reliable, and not greatly affected by uncontrolled body movements. We are testing the validity of the methods by analyzing data from a study of yohimbine, a drug that increases parasympathetic activity. We plan to use the technique on data from patient studies.

C. Interaction of Nature and Nurture in Disordered Behavior

1. Studies of Heredity and Environment in Schizophrenia

The project is composed of the following studies:

a. An intensive multidisciplinary study of a family with monozygous quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome.

The first study of this family was completed and published in a book form in 1963. We have continued our contacts with this family to follow the clinical course of these women and to see how the course is related to earlier and current life experiences. A second intensive multidisciplinary study of these women was completed in June of 1981, and has been summarized in previous annual reports. As of the present time, one theoretical piece describing nature-nurture issues in the Genain quadruplets has been published in a book on aging and schizophrenia and a second has been published in the *Schizophrenia Bulletin.* In this latter work, the status of the Genains is brought up to date, and a new theoretical view of the relation between early damage to the brain and later experiential factors in the Genains is propounded. A third paper has been published in *Progress in Experimental Personality Research;* it addresses the nature-nurture issue highlighted in the Danish Adoption Study results and in life outcomes of the Genains.

b. Studies of adoptees with schizophrenic parents and their biological and adoptive families.

This represents an updating of work done with a cohort of adoptees and controls that were obtained in Denmark in the 1960's. Although a considerable portion of this work has been published, much of the psychological information was not analyzed. In addition, we now have contemporary assessment of outcome of probands in these studies. A reanalysis of the original case material, which reaffirmed the original finding, has been published. A paper comparing disordered and non-disordered subjects in terms of reported stresses during development is in press in a peer-reviewed journal on experimental personality research (see a. above). The data suggest that intense familial stress during development can interact with a schizophrenic diathesis to produce schizophrenia spectrum illness. In contrast, controls experiencing such stresses remain free from mental illness. This finding appears to be related to the body of work on the construct of "Expressed Emotion" in the family and recidivism in schizophrenic patients. That work indicates that punitive, aggressive home environments are associated with more rapid return to hospitals in schizophrenic patients.

c. A study of children of schizophrenic and control parents reared in town or kibbutz in Israel.

This study, begun in 1962, has involved a multidisciplinary (psychological, psychiatric, neurological) examination of a cohort of 50 children with schizophrenic parents (index cases) together with 50 matched controls. Half of each group was raised on a kibbutz, and half was raised with their nuclear families in cities and towns in Israel. The children were seen three times: once in 1966-67, when they were about 10-11 years of age, once in 1973 when they were about 17, and in 1981 when they were in their mid-twenties. The results of the 1981 study suggest that being raised in a kibbutz environment is more likely to lead to major psychopathology, given a schizophrenic diathesis, than is growing up in the nuclear family

within a city environment. A surprising finding was the large incidence of affective disorders, particularly in the Kibbutz-Index group. Another major followup of the Israeli cohort has now been completed. It includes measures of psychiatric status, behavior on cognitive and attention tests, reanalysis of parental hospital records, reinterviews of the surviving parents, construction of family histories (to address the question of genetic vs. sporadic schizophrenia) and a survey of the status of the siblings of the index cases. At this time, approximately 85 of the original, surviving and traceable 97 subjects have been reinterviewed. (Thirty-five of the surviving parents have been seen, as well). There is no suggestion of an increase in the number of study subjects that have developed schizophrenia since the 1981 assessment. However, there appears to have been an increase in the number with affective disorders, in both Index and Control cases, although more cases are found in the Index groups. The previous finding that psychiatric disorder appears earliest in the Kibbutz-Index cases is still valid. Approximately 65 subjects have been tested with a neuropsychological test battery and the results have been analyzed. The results indicate that the Index cases perform more poorly than Controls in attention tests. We have also found that there is an excess of Axis I disorders (including the schizophrenia spectrum disorders) among the Kibbutz-Index cases.

The results of the several followup investigations of the Israeli children (now in their mid-thirties) are being prepared for a special issue of the *Schizophrenia Bulletin*. Most of the reports have been written and the editorial review process is underway.

The objectives of all of these projects are to understand how hereditary and environmental factors interact to make for schizophrenic outcomes of varying types and degrees.

d. Studies of neuropsychological (attention) test profiles in schizophrenia patients and their first-degree relatives in Western Ireland.

County Roscommon, in the Western part of Ireland, is the site of an ongoing family study of schizophrenia. In the course of this study, we measured several attentional functions in a group of subjects with schizophrenia, their first degree relatives and age-and education matched controls. The total number of subjects studied is now 112. The results indicated that the schizophrenics performed significantly more poorly on the tests than control subjects, and that the scores of the relatives fell somewhere between those of the other two groups. The test results also showed that the best statistical separation among the groups w on those tests that measured the capacity to focus and respond briskly (e.g., the Trail Making Test) and the capacity to sustain attention (e.g., versions of the Continuous Performance Test). We interpret the results to mean that impaired attentive functions are part of the schizophrenic diathesis and suggest that certain tests may be particularly useful in future research on genetic linkage in the disorder.

A manuscript describing this work (as well as a review of the relevant literature) has been accepted for publication in the *Journal of Psychiatric Research*.

2. Studies on Etiological Factors in Schizophrenia

Studies of the occurrence of mental illness in families have been useful in identifying

familial forms of the illnesses and in the development of hypotheses regarding the form and strength of genetic and environmental factors in etiology. Where these major variables are separated by the process of adoption, specific etiologic hypotheses can be tested separately and in combination. A total national sample of 14,500 adult adoptees in Denmark, including 76 who have developed schizophrenia, provide the basis of one phase of this research; the other phase is represented by schizophrenic patients and their families residing in Roscommon County, Ireland. With respect to the Danish cohort, work has now been completed on an analysis of the outcome of the "provincial" sample. This consists of a group of schizophrenic adoptees raised in the region outside of Copenhagen. As in the case of the Copenhagen sample, relatives of index cases were found to have significantly more schizophrenia (latent or chronic) than relatives of controls. A manuscript reporting this work is in press in the *Journal of Psychiatric Research.* Additional work with this sample is now focused on differentiating familial from non-familial schizophrenia and on the behavior genetics of schizophrenia spectrum disorders.

3. Genetic Factors in Response to Alcohol

This project, which is being executed by Dr. Gabbay, seeks to evaluate the relative contributions of heredity and environmental factors in response to alcohol. To date this project has involved completion of a survey of drinking behavior in a large cohort of mono- and dizygotic twins.

ANNUAL REPORT OF THE LABORATORY OF SOCIO-ENVIRONMENTAL STUDIES
NATIONAL INSTITUTE OF MENTAL HEALTH

Carmi Schooler, Ph.D., Acting Chief

October 1, 1991 through September 30, 1992

The research of the LSES examines how social-structurally determined environmental conditions, on the one hand, and psychoneurological processes, on the other, affect normal and abnormal psychological functioning across the life span. This approach has also led to basic research on the nature of certain cognitive and social-psychological processes-- as well as to some work on basic neuropsychological and sociological issues.

The two aspects of psychological functioning that are the major foci of our current interest-- cognitive processing and self-direction/autonomy-- derive from earlier work of the Laboratory. LSES research on the effects of complex environments (i.e., those with diverse ambiguous stimuli requiring many decisions) on normal individuals has shown that exposure to environmental complexity increases both intellectual flexibility and self-directedness of orientation to self and society. In contrast, Laboratory research on schizophrenia suggests that exposure to complex environments or to relatively intense interpersonal situations produces a decrement in intellectual functioning in schizophrenics.

The research carried out this year is of three general types: I) sociological and social psychological research on the psychological effects of social-structurally determined environmental conditions; II) cognitive studies seeking to elucidate the psychological mechanisms through which the environmental conditions have their effects; III) an extensive study of social, cognitive and ocular interference in schizophrenia.

PSYCHOLOGICAL EFFECTS OF SOCIAL STRUCTURALLY DETERMINED ENVIRONMENTAL CONDITIONS

The presently ongoing research on this project can be divided into three categories: A) continued analyses of already collected occupation study data, with particular emphasis on the socio-environmental determinants of the intrafamilial transmission of cognitive and non-cognitive aspects of psychological functioning; B) developing and carrying out a new follow-up survey of the respondents in the LSES occupation study to examine environmental effects on psychological functioning in older people; C) initiation of a new series of analyses on social background, social conditions and psychiatric illness.

In their continued analyses of occupational study data, Kohn, Schooler and Schoenbach are modeling the relative effects of parents' background, social status and occupational self-direction on the transmission of intellectual flexibility, distress, self-directed orientations and values from parents to their children. Schooler and Schoenbach are also working on even more complex models for the U.S. families, which attempts to examine how parents' supportive behavior and controlling behavior affect children's psychological functioning.

Although analyzing the data already in hand is challenging, we can't help but be excited at the prospect of being able to collect new data. With the strong encouragement and excellent prospect of receiving very substantial support from the Behavioral and Social Research Program of the National Institute on Aging, we are far along in the planning of a longitudinal study of the reciprocal effects of social environments and psychological functioning in older people. The empirical basis of this investigation would be a resurvey of the 1974 respondents. Through a contract let to the Equifax Co., and largely funded by NIA, we have now succeeded in relocating 650 of these households.

This year, in addition to relocating 95% of our 1974 respondents, we have developed and successfully pretested an initial version of our interview, which deals with a variety of important issues in aging such as the socio-environmental determinants of effective intellectual functioning and coping with health and financial problems. This initial version also gets information about the occupational conditions of those who are still employed, measures the nature of housework, voluntary and leisure time activities and provides measures of the relevant psychological variables. We are also carrying out experiments aimed at determining the best way to have the respondents recall and provide information about what happened in their lives since we last saw them. On the basis of what we have accomplished we are now in the process of seeking financial support from NIA for the full implementation of our proposed study.

A renewed area of interest at the LSES is the relation between environment and psychopathology. The use of structural equation modeling and other maximum likelihood methods (e.g. LISREL, Latent Class Analysis) for determining latent structures and for causal modeling is propelling new developments in this area. A particular advantage of structural equations modeling for this kind of analyses is that it allows for the simultaneous test of hypotheses including sociological, psychological, and biological variables, and their relationships. Three research projects in this area have been initiated this year at the LSES by Carles Muntaner with the collaboration of Schooler.

One project examines patterns of social drift and mobility in DSM-III disorders by reexamining questions about the relationship

between social status and occupational conditions, on the one hand, and the etiology and symptomatology of psychiatric illness, on the other. It is based on a large sample (n=1600) of first admission psychotic patients from 15 psychiatric institutions in the greater Baltimore/Washington Area. A related study, based on the same sample, has the clinically and socially important goal of identifying the psychosocial and clinical factors associated with family and hospital violence in different psychotic conditions.

The third study examines the relationship between occupational environments and substance. It is an extension of the Karasak Demand/Control/Support model of the work environment (developed partly on the basis of the Kohn-Schooler occupational research) to drug abuse. Incidence data from the Epidemiological Catchment Area Study (Robins et al. 1981) is being used to provide cases and non-cases matched on non-work environmental variables.

COGNITIVE STUDIES

In the cognitive research project, Drs. Caplan and Schooler began by trying to explain Kohn & Schooler's finding that people who were employed in more complex occupations demonstrated greater flexibility of thought even in tasks and contexts that were not job-related. In doing so, we have developed a model based on the principle of transfer-appropriate processing. Models of transfer-appropriate processing posit that memory retrieval is best when the processing occurring at the time of retrieval is the same kind of processing that occurred at the time information was encoded. In this project, we have tested the hypothesis that learning by analogy, which presumably involves the abstraction of information common to two domains, should be best when encoding has been complex; in contrast, we have hypothesized that learning without analogy, which does not require such abstraction, should be best when encoding has been simple. To date, we have obtained such findings using two very different experimental paradigms: computer learning and text comprehension.

Another way of investigating transfer-appropriate processing with these analogy manipulations involves comparing younger and older adults. A number of researchers have suggested that older adults have difficulty engaging in "deep" conceptual processing - in other words, in abstraction-based processing. If this is the case, then training older adults with analogies and/or models may not help their performance, and could, in fact, impair it; our previous results are consistent with this hypothesis. Recently, Drs. Lipman and Caplan have extended this theoretical approach to a third paradigm: the study of the acquisition of sequentially-presented macro-spatial information, or route learning. In a recent study, they hypothesized that map-like schematic diagrams of the shape of the route would impair older adults' learning configural aspects of a route. The findings

supported this hypothesis, and are currently in press. Drs. Lipman and Caplan are currently investigating the effects of different kinds of map and diagram in a study now underway.

Central to discussions of the usefulness of episode-based and abstraction-based processing is basic research regarding the role of categories in generalization and transfer. Much current research in the area of category representation focuses on evaluating abstraction-based (i.e., feature theories, prototype theories) and episode-based theories (i.e., exemplar theories) of category representation. These issues comprise a major question in work on natural language categories being conducted by Dr. Caplan in collaboration with Dr. Robin Barr of the National Institute on Aging. They have developed a model of natural language categories which assumes that category representation involves the abstraction of features across category members. In particular, the model distinguishes between two kinds of feature that may be involved in representations of categories (intrinsic and extrinsic features), and have proposed that the way in which people reason about a category is profoundly affected by whether the representation of the category is primarily intrinsic or extrinsic. For example, the model can explain many findings of other investigators, including a number of aspects of categorization phenomena that were formerly believed to be inconsistent with a feature-based model of categorization. A recent modification of the model to include inter-category differences in feature necessity, with supporting evidence, was recently published.

OCULAR, COGNITIVE AND SOCIAL INTERFERENCE IN SCHIZOPHRENIA

This aspect of the LSES's research program consists of a series of interconnected psychological experiments, carried out on the same subjects, that examine the nature of four frequently cited psychological abnormalities in schizophrenia: (I) an inability to focus attention on task relevant aspects of the environment (II) anomalies in smooth pursuit eye movements (III) prolonged reaction times irrespective of temporal uncertainty or predictability of the forthcoming event and (IV) severe social dysfunctions as manifested by tendencies toward social withdrawal and cognitive disruption when dealing with social phenomena.

Although these abnormalities are not all necessarily related, by examining them on the same subjects we hope to test a set of hypotheses that predict the existence of empirical relationships among them stemming from their being, at least in part, manifest indicators of more basic disturbances in schizophrenics' psychological and prefrontal functioning.

Completing our full experimental series takes about 15 hours. As of now we have run over 65 subjects through our basic experimental protocol. These subjects have come from the NIMH

Neuropsychiatric Hospital at Saint Elizabeths, from the inpatient wards at St. Elizabeths and from the Area D outpatient center.

Although this LSES experimental research program on schizophrenia also includes various ancillary experiments, it is worth noting that this experimental psychopathology research focuses on the same questions as the Laboratory's sociological research-- How environmental complexity, intellectual functioning and the social environment of individuals are causally related.

CONCLUSIONS

The scope of the research now being carried out by the LSES represents a substantial broadening of its focus from what it was a decade ago. This summary and the individual project reports provide a full accounting of our present purposes, recent accomplishments, ongoing projects and future plans. I believe that they also show that we are not only quite productive, but unusual in the number of levels of phenomena we investigate, the variety of our research techniques, and the span of the life course we examine. Our research also encompasses the gamut of normal and abnormal psychological functioning, dealing with both basic and applied issues. Despite this unusual scope, our program is coherent. Our concern with the two aspects of psychological functioning that are the major foci of our interest-- cognitive processing and self-direction/autonomy-- stems from our earlier research on how they are causally interconnected with each other and with socially and cognitively complex environmental conditions in both normal and mentally ill individuals.

Despite the diversity of our research, we remain strongly connected to the relevant professions and disciplines; what we do is firmly grounded in their respective theories and methodologies. Hopefully, we have shown how such an interdisciplinary and cross-field approach can decrease the likelihood that relevant empirical and theoretical considerations that seem unimportant from the vantage point of some particular field will be overlooked, while increasing the likelihood that innovative methodological techniques developed in one field will be applied to another. We hope that it is also apparent that a similar kind of cross-fertilization takes place through our study of both normal and abnormal psychological functioning.

Annual Report of the Laboratory of Cell Biology
National Institute of Mental Health
Michael J. Brownstein, M.D., Ph.D., Chief
October 1, 1991 - September 30, 1992

INTRODUCTION

Receptors and transporters

A second vasopressin receptor--the V2 subtype--has been cloned. This receptor's mRNA has only been detected in the kidney to date; it mediates the antidiuretic action of vasopressin. The mRNA encoding the V1a receptor, on the other hand, is in several peripheral organs and in the brain as well.

The gene that encodes the V2 receptor is found on the long arm of the X chromosome. A defect in the coding region of this gene--in the first pedigree studied--is responsible for X-linked nephrogenic diabetes insipidus.

A novel somatostatin receptor, the third described, has been cloned and characterized. This receptor shows marked preference for somatostatin 28 as opposed to somatostatin 14. Several additional orphan receptor clones have been isolated and are being examined.

A second 5HT-1D receptor has been cloned and characterized. This is the target of a novel class of drugs used to treat migraine.

A second glycine transporter cDNA has been isolated. The mRNA encoding this transporter seems to arise from the same gene as the mRNA encoding the glycine transporter described by P. Hartig and his coworkers. The two transporters have different N-terminal sequences and different tissue distributions. Inhibitors of these transporters may be useful for treating spasticity and may be useful as sedatives or anticonvulsants.

The CNS vesicular monoamine transporter has been cloned. This transporter appears to span the vesicular membrane 12 times. Unlike the monoamine uptake proteins, which are sodium cotransporters, it is a proton antiporter and is inhibited by reserpine and tetrabenazine.

A method has been developed for identifying agonists or antagonists acting on cloned receptors that increase intracellular calcium.

The cellular localizations of histamine, dopamine, and acetylcholine receptors in the stomach and duodenum have been examined. Unexpectedly, these receptors are all found on immunocytes, not epithelial cells.

Stimulation of the muscarinic M1, 3, and 5 receptors results in simultaneous increases in phospholipase A_2, phospholipase C, phospholipase D, adenylate cyclase, tyrosine kinase, and calcium influx. Calcium influx and tyrosine kinase activation occur at agonist concentrations one-one hundredth as great as those required to see other second messenger effects. Muscarinic receptor-induced tyrosine phosphorylation was calcium dependent; phospholipase C-gamma was one substrate.

The muscarinic receptors M1, 3, and 5 have been found to induce a morphological change and to have a tumor suppressor effect when they are expressed in CHO cells. These effects seem to result from a calcium-dependent increase in tyrosine phosphorylation.

Unlike the other G_i-coupled receptors studied to date, the cannabinoid receptor does not augment ATP-stimulated phospholipase A_2 activity. High concentrations of cannabinoids caused arachidonic acid release, increased intracellular calcium, and reduced arachidonic acid uptake in a receptor-independent manner.

G-proteins

G-protein $\beta\gamma$ dimers have been produced using the baculovirus

expression system. Soluble and membrane-bound dimers are produced; the latter have prenylated γ subunits. Both forms are active. They facilitate the interactions of $GTP_{\gamma}S$ and pertussis toxin with $G_t\alpha$.

The forskolin-resistant mutant of the Y1 cell line has been studied in detail. The results of these studies suggest that an alteration in the $\beta\gamma$ dimer may be responsible for the functional defect in the cell line.

Polyclonal antisera were used to study the localization and development of G-proteins and phosphodiesterase in the rat retina. Transducin and phosphodiesterase were detected in immature photoreceptor cells in postnatal day 9 pups. In younger animals, no expression was detected. In adult animals, transducin and phosphodiesterase were also exclusively found in the outer retinal segment. G_o, on the other hand, was localized to the plexiform and ganglion layers. The small molecular weight GTP bind protein, G_p, was found in the synaptic layer.

Molecular basis for neuropeptide/neurotransmitter specificity in the diffuse neuroendocrine system

Both positive regulation of enkephalin expression by protein kinase A and calcium pathways, and negative regulation of enkephalin expression by the protein kinase C pathway, occurs via a direct effect on the transcription of the enkephalin gene in primary bovine chromaffin cells. The bovine enkephalin gene has been sequenced and is virtually identical to the rat and human genes in the region of its PMA-response element (for which positive regulation by PMA has been noted), suggesting that the PMA response element of this particular gene can confer both positive and negative regulation as a function of cell type. Tissue-specific expression of enkephalin in chromaffin cells, and its up-regulation by cell depolarization, correlates with DNase hypersensitivity in the first intron, and proximal promoter region, of the enkephalin gene, implicating these regions in the binding of cell-specific transacting protein(s), subserving cell-

specific enkephalin gene expression, to the enkephalin gene in bovine chromaffin cells. Primary cultures of bovine chromaffin cells can be used to study the cell-specific expression of other neuropeptide/neurosecretory genes, including enkephalin, galanin and chromogranin A, via transfection of promoter/reporter gene constructions. Each of these genes is specifically expressed in bovine chromaffin cells, but each has a unique mode of cell-specific regulation including cell-specific derepression mediated by 5'distal gene elements (chromogranin A), cell-specific alteration in transcription in response to electrical stimulation (enkephalin) and cell-specific enhancement of gene transcription after activation of protein kinase C (galanin). Neuropeptide and secretory protein biosynthesis is under independent regulation in chromaffin cells: biosynthesis of the secretory proteins chromogranin A and B is insensitive to cell depolarization or protein kinase signalling, reflecting their potential role in constitutive vesicular biogenesis. Chromogranin A, a secretory protein potentially involved in endocrine vesicle biogenesis, is expressed at high levels in non-basilar keratinocytes (which also contain distinct vesicular organelles), in primate and rodent skin. Studies of the catalysis of secretory vesicle morphogenesis by expression of a chromogranin A expression plasmid in non-endocrine, non-vesicle-containing recipient cells is underway.

Chronobiology

Dr. Zatz and his coworkers have shown that cyclic AMP is a key regulator of melatonin production by chick pineal cells. Light and L-type calcium channels act in part through cyclic AMP, but the circadian pacemaker does not.

Gene expression

A novel POU protein, RHS2 (Brain-4) has been cloned. The distribution of the mRNA encoding this transactivator has been studied in detail in hopes of obtaining clues about the genes it regulates.

The developmental expression of mRNAs encoding the four thyroid hormone receptor subtypes has been determined. These studies have revealed much about the cellular targets of thyroid hormone in fetal development.

Developmental biology

Transgenic mice carrying a bacterial ß-galactosidase gene (lacZ) under the control of the retinoic-acid-receptor-ß-2 (RARß2) gene promoter were used to study the effects of retinoic acid (RA) on the transcriptional activity of this promoter in vivo. These studies suggest that mesoderm transformation, possibly by ectopic induction of the RARß2 gene, plays an important role in the observed terotogenic effects of RA. RA treatment at day 8.5 to 10.5 induced expression of the transgene in a segmented pattern reminiscent to that of pair rule genes in the forebrain, midbrain, and hindbrain. The characteristic appearance of transient bulges during neural tube development has been interpreted as an indication of an intrinsic segmented structure. However, supportive molecular and cellular evidence exists only in the hindbrain region. Therefore, the CNS-segmentation remained controversial especially in the most anterior regions. Data collected to date strongly suggest that anterior regions of the CNS are truly segmented.

The 5'-untranslated region of the RARß2 gene contains several short open reading frames. These open reading frames have been shown to play an important role in the regulation of the tissue specific expression pattern of the RARß2 gene. Several genes which are supposed to be involved in the regulation of cell growth and differentiation have similar open reading frames in the 5' untranslated region. A common factor might act on these genes to regulate their tissue specific expression at the posttranscriptional level.

Tetanus toxin is produced by Clostridium tetani. Upon uptake into neurons it inhibits the calcium-stimulated release of neurotransmitter by a yet unknown mechanism. Several transgenic lines have been established which carried the tetanus toxin gene

under the control of either: (1) an SV40 early gene promoter; (2) the promoter of the pro-opiomelanocorticotropin gene (POM-C); or (3) the L7 gene promoter. All expressing transgenic males, with the notable exception of one founder animal, were sterile regardless of the promoter used. These results show for the first time that tetanus toxin can effect cells other than neurons. Calcium-dependent exocytosis may occur in Sertoli cells and play an important role in sperm cell maturation.

Structure and function of the basal ganglia

D1 and D2 receptors have been activated concurrently, and interactions among striatal neurons have been examined. The D1 and D2 receptors seem to be present on separate populations of cells, and the effect of D1/D2 receptor stimulation is a synergistic one.

Mechanical, thermal, and optical correlates of neuronal excitation

Using a thin film of poly(vinylidene fluoride) a sensitive thermal detector with a time-resolution of about one millisecond has been constructed. The detector has permitted the time-course of heat generated by the sciatic nerve following excitation to be measured. The results have been interpreted in the context of a divalent-univalent cation-exchange theory.

The garfish olfactory nerve has been found to swell at the site of electrical stimulation simultaneously with or preceding the optical changes observed there. This suggests that there is a rise in the water content of the superficial layer of the nerve fiber which accounts for the optical signals.

Viral and cellular factors governing retroviral infection

A murine ecotropic receptor has been cloned and the amino acid residues critical for binding and entry of ecotropic vectors have

been mapped.

Fusion of the ecotropic virus prior to entry into the cell and the fusion process responsible for virally induced cytopathology (i.e., formation of multinucleated giant cells) have been shown not to be linked processes. Therefore, it may be possible to inactivate the cytopathic region of the viral envelope without affecting viral entry.

Using oligonucleotide probes based on the structure of the gibbon ape leukemia virus (GaLV) receptor, the latter has been shown to be restricted to specific cells in different stages of development. Therefore, it may prove possible to use GaLV retroviral vectors to introduce genes into a restricted subset of cells on tissues.

AIDS and AIDS dementia

The role of the CDR2- and CDR3-like domains of CD4 in binding to the HIV-1 gp120 envelope glycoprotein has been further examined with monoclonal antibodies to the CDR3-like domain and derivatized peptides from the CD4(81-92)[CDR3-like] region of CD4. Antibodies to the CDR2- and CDR3-like domain of CD4 can bind simultaneously to discrete epitopes on the CD4 molecule, yet both block gp120 binding to CD4, demonstrating separate binding sites for gp120 within the CDR2 and CDR3-like domains of CD4. CDR3- and CDR2-directed antibodies, but not soluble CD4, at concentrations of 1-10 µg/ml, block in vitro infection by primary/clinical pediatric isolates of HIV-1. CD4(81-92) peptides block infection of some but not all primary/clinical pediatric isolates of HIV-1. Thus, the interaction between CD4 and the HIV-1 gp120 envelope glycoprotein involves multiple sites on both molecules, and a processive series of binding events leading to virus entry and syncytium formation. While CDR2 and CDR3 binding sites both appear to be necessary for attaining high-affinity interaction between 'wild type' CD4 and gp120, the relative contribution of each site to the stability of the final complex may be quite disparate, and may vary between clinical and laboratory, as well

as macrophagetropic and lymphocytotropic, isolates of HIV-1. The interaction between CD4 and the HIV-1 gp120 envelope glycoprotein involves multiple sites on both molecules, and a processive series of binding events leading to virus entry and syncytium formation. Physical and functional mapping of gp120/CD4 interaction, particularly between CD4 and envelope protein from primary/clinical HIV-1 isolates, forms a basis for design of peptide antiviral, passive immunization, and active immunization strategies for blocking HIV-1 infection and post-infection pathogenesis.

Infection of juvenile rhesus macaque monkeys results in profound motor and cognitive deficits in some of the animals. Early motor/cognitive impairment of SIV-infected rhesus macaques is correlated with a poor prognosis for surviving immune disease, as reported for pediatric AIDS patients with progressive encephalopathy. Diffuse regional cerebrocortical astrogliosis and increased expression of somatostatin mRNA have been noted in the frontal and parietal cortices of SIV-infected monkeys. Systematic studies will reveal whether or not regional patterns of astrogliosis and neuropeptide dysregulation are markers for, or contributory to, motor and cognitive impairment accompanying SIV infection in the rhesus macaque monkey.

Retrovirally tagged bone marrow stem cells introduced into lethally irradiated donor mice can be found, albeit rarely, in the central nervous system. Attempts to increase brain targeting of retrovirally transduced hematopoietic cells in mouse models of encephalitis and blood-brain-barrier compromise, are underway, as are attempts to use primate retroviral vectors to introduce exogenous genes encoding marker and potentially therapeutic proteins into bone marrow cells of rhesus macaque monkeys for parallel experiments in this nonhuman primate model for immunodeficiency disease.

Like heparin/heparan sulfate, Sp54 is known to inhibit infection of cells by HIV in vitro. Sp54 has been fractionated and the fractions have been tested in assays for "cell entry" and cell fusion.

Sulfated ß-cyclodextrin has been fractionated and the fractions tested for their ability to inhibit the growth of Kaposi sarcoma cells in vitro.

The interaction of suramin, a chemotherapeutic agent, with bFGF has been studied. Suramin seems to bind close to the binding site on bFGF's signal transducing receptor.

Summary of Annual Report of Laboratory of Cerebral Metabolism
National Institute of Mental Health
October 1, 1991 through September 30, 1992
Louis Sokoloff, M.D., Chief

The Laboratory of Cerebral Metabolism (LCM) consists of two Sections, the Section on Developmental Neurochemistry (SDN) and the Section on Clinical Brain Imaging (SCBI). The Section on Developmental Neurochemistry engages in basic research in neurobiology, mainly neurochemical and neurophysiological, and is renowned for its development and applications of methods for determining local rates of physiological and biochemical processes *in vivo* in brain without direct invasiveness of the brain. Two of the methods that it developed, the methods for the measurement of local cerebral blood flow and local cerebral glucose utilization have been adapted for use in man with Positron-emission tomography (PET). In fact, it is these methods that established the usefulness of PET and led to the current popularity of "Brain Imaging" in neurobiological research. At the present time, these are the only two truly authenticated quantitative brain imaging methods available in the PET field. The Section on Clinical Brain Imaging utilizes these methods in PET studies of nervous and mental disorders in man and in animal models of human disease. The SCBI also works on the development and evaluation of radioactive compounds and ligands labeled with positron-emitting isotopes that might be useful for studying local rates of neurotransmitter synthesis or receptor densities in the brains of human subjects.

Section on Developmental Neurochemistry

Louis Sokoloff, Chief

This Section's research is still predominantly concerned with the development, validation, refinement, and applications of methods for measuring local rates of physiological and biochemical processes in the nervous system. The initial basic work is always first done in animals with quantitative autoradiography, a technique first introduced by this Section, and then, whenever possible, adapted, in collaboration with others with the necessary resources, for use in man with external detection by single photon emission tomography (SPECT) or PET. For example, the two currently most extensively used methods in man with PET are the [^{18}F]fluorodeoxyglucose (FDG) method for measuring regional cerebral glucose utilization and the [^{15}O]H_2O method for measuring regional cerebral blood flow; these are direct adaptations of the Section's autoradiographic [^{14}C]deoxyglucose (DG) and [^{14}C]iodoantipyrine (IAP) methods, respectively. Because the Section originated these methods and was instrumental in their adaptation to human use with PET, it maintains a proprietary interest in their proper use and application by the many laboratories throughout the world that use and often abuse them. The Section, therefore, feels obligated to monitor and critically review modifications of the methods and to correct them if necessary.

For example, the theory of the [^{14}C]DG method was based on the assumptions of a homogeneous tissue compartment and no loss of product. On this basis a two compartment kinetic model was designed which was described by an operational equation with three rate

constants (3-K model). The requirement of a homogeneous tissue could be reasonably satisfied in animals by the use of autoradiography with its fine resolution (approximately 200 μm) and the extension of the duration of the experimental period after the intravenous pulse of [^{14}C]DG. The assumption of no product loss was validated by experimentally determining the maximal permissible duration of the experimental period before effects of product loss became detectable and limiting the experimental period to that time; in the rat this was found to be 45-50 minutes after the pulse. When the DG method was first adapted for use in man with and PET and [^{18}F]FDG, the PET scanners available at that time had only single rings and were inefficient and slow. It was, therefore, necessary to prolong the scanning period for at least 2-3 hours, long enough for loss of labeled products of [^{18}F]FDG phosphorylation to occur. A fourth rate constant e.g., k_4^*, 4-k Model) was, therefore, added to the model to correct for any possible loss of labeled product. The incorporation of a k_4^* into the model does indeed improve the non-linear least squares fit of the model equation to the entire time course of ^{18}F accumulation in brain, and this has generally been interpreted as proof of product loss due to glucose-6-Pase (G-6-Pase) activity in brain. Previous work in this Section had showed, however, that product loss is insignificant at shorter times (e.g., less than one hour); it needs to be taken into account only at longer times after injection of the tracer. Nevertheless, because the use of k_4^* improves the fits, many still believe that there is significant product loss and continue to use the 4-k model, even though much shorter scan times are now possible with the fast modern PET scanners.

Work in this Section during the past couple of years has demonstrated that the use of the 4-k model with the shorter procedures now possible with PET scanners actually leads to erroneous results because of the misapplication of a model and operational equation designed for a homogeneous tissue to heterogeneous tissues. K. Schmidt first carried out simulation studies that clearly showed that heterogeneity fully accounts for the findings of significant positive k_4^* values and better fits to the dynamic PET data with the 4-k model, even when there is no product loss. She showed, moreover, that the application of the k_4^* model to heterogeneous tissues, which is a prevalent practice in the PET field, leads to erroneously high values for glucose utilization when product loss is negligible as it is with the 45-60 minute scans now used with the modern PET scanners. She also extended the original DG model, which assumes no product loss, to heterogeneous tissues, and this tissue heterogeneity model (e.g. TH Model) fits the dynamic scans as well or better than the k_4^* model. These simulation studies were published in 1991 in the *Journal of Cerebral Blood Flow and Metabolism*. In the past year the results of the simulation studies have been verified directly by comparable analyses with actual PET data obtained from normal subjects in collaboration with G. Lucignani (a former Visiting Fellow in the Section) and the PET group at San Raffaele Hospital in Milan, Italy. The problem is that the spatial resolution of even the most modern PET scanners is relatively poor (e.g., 5-6 mm), and the resulting partial volume effects make it impossible to satisfy the assumption of a homogeneous tissue required by the 3-k and 4-k models when they used with dynamic PET data acquired over the full time course of uptake of tracer by the brain. On the other hand, both the TH Model and the 3-k Model, when used by the originally prescribed procedure (i.e., no dynamic scanning but a single PET scan performed late after the pulse of tracer when the

effects of heterogeneity are reduced to negligible levels) provide accurate estimates of the true regional rates of glucose utilization within the brain. The study with the actual PET data has been published this year in the *Journal of Cerebral Blood Flow and Metabolism*. K. Schmidt also, has analyzed the PET data from the normal subjects to define the optimal model and duration of the experimental period in man. The results indicate that in man there is no evidence of product loss until about 120 minutes after the pulse in contrast to the 50-60 minutes in the rat. The optimal duration of the procedure in man was found to be 60-90 minutes. A manuscript on these studies has recently been submitted to the *Journal of Nuclear Medicine*.

Studies have been continued on the development of a $^{3}H/^{14}C$ double-isotope quantitative autoradiographic DG method. The purposes are two-fold: 1) to apply the DG method to measure local cerebral glucose utilization ($lCMR_{glc}$) simultaneously with the measurement of the local lumped constant with 3-O-methylglucose; 2) to determine $lCMR_{glc}$ by means of the DG method twice in the same animal by sequential determinations alternately with ^{14}C- and ^{3}H-labeled DG.

1) Under normal physiological conditions it is not necessary to redetermine the local lumped constant simultaneously with each determination of $lCMR_{glc}$ because the lumped constant is uniform throughout the brain and stable so that the values previously determined and reported by this Laboratory can be used. The lumped constant can, however, change in pathological states and vary locally within the brain with focal pathology. In such cases a method to determine the local lumped constant is necessary, and preferably it is one that can be used simultaneously with the measurement of $lCMR_{glc}$. G. Dienel, K. Mori, N. Cruz, and T. Nelson of our Section previously carried out extensive and difficult *in vivo* biochemical experiments to acquire the data needed to develop and validate the theoretical basis for the use of labeled 3-O-methylglucose to determine the local lumped constant autoradiographically. The data needed were measurements *in vivo* of brain and plasma glucose, DG, and methylglucose concentrations under conditions in which the brain has been brought into steady states for all of them. They collected all the experimental data needed to relate the local lumped constant quantitatively to the local tissue glucose concentration and also the local glucose concentration to the local 3-O-methylglucose concentration in the tissue. These studies were published in 1991 in the *Journal of Cerebral Blood Flow and Metabolism*. In these experiments brain tissue glucose concentration was varied by clamping the plasma glucose levels at various levels from hypoglycemia to hyperglycemia, i.e., by altering glucose supply to the brain. There are theoretical reasons why these may be different when glucose concentration in brain tissue is changed as a result of altered glucose utilization rather than glucose supply. Dienel, Cruz, and K. Adachi are, therefore, carrying out experiments in which brain glucose concentration is altered locally by focal changes in $lCMR_{glc}$ upward or downward by focal topical applications to the exposed cerebral cortex of convulsant drugs, e.g., penicillin, bicuculline, barbiturates, etc. These very difficult experiments are almost completed. The results thus far show that the glucose concentration in tissue is altered when glucose utilization is changed, but apparently not enough to have major effects on the lumped constant. One interesting finding of these studies is that there

are regulatory mechanisms that zealously guard the brain against changes in blood glucose content and maintain a more or less constant rate of energy metabolism in the face of a widely fluctuating supply of the brain's essential substrate, glucose. This may be a residual trait from the time of the hunter-gatherers when the ability to hunt for food would not be impaired by prolonged food deprivation. The ultimate goal of these studies is, however, the perfection of a method with which by administering both DG and 3-O-methylglucose, one labeled with ^{14}C and the other with ^{3}H, and measuring plasma glucose concentration enzymatically and labeled DG and 3-O-methylglucose concentrations in plasma by double-label liquid scintillation spectrometry, and local tissue ^{14}C and ^{3}H concentrations by double-label quantitative autoradiography, $lCMR_{glc}$ can be computed simultaneously with the local lumped constant for the identical local regions.

2) The $^{14}C/^{3}H$ double label method for two sequential determinations of $lCMR_{glc}$ in the same animal is of importance for metabolic mapping in valuable animals trained to carry out a behavioral task. Normally, the DG method allows only a single determination of $lCMR_{glc}$ in an animal without the possibility of its survival because its brain is removed for autoradiography at the end of the experimental procedure. The sequential double label technique would allow sequential determinations in the same animal during normal control and experimental conditions. Like the project on the determination of the local lumped constant, this one is also absolutely dependent on the availability of a double label quantitative autoradiographic technique. H. Nakanishi, C. Smith, C. Kennedy, C. Dermon, and B. Agranoff (Fogarty Scholar) have been struggling to develop such a technique. The problem is that the ß-radiation of ^{3}H is so weak that differential self-absorption within the cut brain sections alters the quantitative relationship between the actual tissue concentration and the optical density of the film differently for each structure of the brain visualized in the autoradiograms. The difference is primarily between gray matter and white matter, and the structural variability reflects the various degrees of admixture of gray and white matter in the various components of the brain. The strategy is to find a solvent system and to design a procedure that extract the lipids from the brain sections without washing out significant amounts of the water-soluble labeled compounds. Results thus far indicate that anhydrous hexane may meet these requirements, but the results are variable, and the basis of this variability is under investigation. As soon as the problem of the variability is solved, calibration of the ^{14}C- and ^{3}H-labeled methylmethacrylate autoradiographic standards needed for the quantitative autoradiography will be completed.

There is, however, an additional problem with sequential double label DG studies. Because loss of product occurs in rats by 60 minutes, the duration of the experimental period should be limited less than 60 minutes and both the first and second procedures should be completed within that time interval. A considerable amount of DG labeled with the first isotope is retained, however, in the plasma and tissues by the end of the first procedure. If the conditions of the animal in the first and second periods are identical, then there is no problem. If, however, conditions of the animal are different in the second period, as it would be if the method is used to compare control and experimental states, then the residual labeled DG from the first study would then be taken up by brain during

the second period in accordance with the conditions that then pertain. This would obscure the difference between the two states. M. Lyon (Guest Researcher) and C. Smith have demonstrated this dilemma and quantified its magnitude. They administered the pulse of [^{14}C]DG at zero time and initiated continuous electrical stimulation of one sciatic nerve either at zero time or 25 minutes after the pulse; the animals were killed at 50 minutes after the pulse. The results showed that the effects of stimulation on glucose utilization were clearly detectable with the stimulation between 25 and 30 minutes although the magnitude was reduced to 50% of that seen with the stimulation between 0 and 25 minutes. The residual effect, though reduced, was still too great to allow the use of sequential double label DG studies in two different conditions. Lyon and Smith tried to remove the residual labeled glucose of the first experimental period by exchange transfusions near the end of the first period; this did help somewhat but not enough. The results of these studies are in press in *Neuroscience Letters*.

In the course of the studies on the local lumped constant G. Dienel and N. Cruz found that [^{14}C]deoxyglucose-6-phosphate (DG-6-P) is further metabolized to a greater degree than generally appreciated. For example, it can enter the pentose phosphate shunt pathway. With 1-^{14}C-labeled DG, the radioactive DG most commonly used, some ^{14}C could possibly be lost and account for the product loss observed with prolonged DG procedures. They, therefore, compared the local rates of cerebral glucose utilization in rats measured with 6-^{14}C- and 1-^{14}C- labeled DG over prolonged experimental periods and found them to be essentially equal, indicating that this pathway does not contribute to the product loss. These studies were recently reported in the *Journal of Neurochemistry*. Dienel and Cruz also found at least 7 non-acidic and acidic secondary metabolites of DG-6-P. Some of these were acid-labile, and special extraction procedures were devised to recover them. These included surprisingly high levels of DG-1-6-diphosphate and DG-1-P, precursors on the way to glycogen and oligosaccharides. Some labeled secondary metabolites appeared in the plasma following [^{14}C]DG administration. They, therefore, examined whether the secondary metabolism of DG-6-P might affect the accuracy of the values for cerebral glucose utilization obtained with the routine autoradiographic DG method; they found the effects to be very minimal because the metabolites in brain turn over very slowly and are not appreciably lost from the tissues during the experimental procedure, and the level of labeled metabolites in plasma is very small compared to the amounts of DG. These results are in press in the *Journal of Cerebral Blood Flow and Metabolism*. Dienel and Cruz are currently examining the possibility that labeled DG could be used to study glycoprotein synthesis. Glycoproteins are, of course, of interest because they are ubiquitous and include many proteins with important functions, such as membrane cell-recognition proteins, receptors, etc. It is difficult, however, to assay the rates of glycoprotein synthesis *in vivo* because conventional precursors, such as labeled glucose or mannose, are also metabolized to amino acids, which are then incorporated into the protein moieties of the glycoproteins. It then becomes impossible to distinguish between labeling of peptide and carbohydrate moieties by quantitative autoradiography. Labeled DG has the advantage over labeled natural precursors because of its more limited metabolism to other compounds. Dienel and Cruz are now examining

the possibility of using labeled DG to study the turnover of the carbohydrate portions of glycoproteins regionally in the brains *in vivo* by means of quantitative autoradiography.

Studies have been continuing on the effects of drugs of abuse on local metabolic processes in the brain. A. Crane Tannenbaum and L. Porrino (Guest Researcher) have completed their studies on the correlation of effects of chronic cocaine administration on behavior and local cerebral glucose utilization, and they are preparing a manuscript for publication. F. Orzi (Visiting Associate), C. Smith, and J. Macedonia have been studying the effects of acute and chronic cocaine administration in rats on local cerebral protein synthesis. The results are currently being subjected to statistical analyses, and though still not complete, there appear to be some very interesting regional effects, particularly in the limbic structures, although additional animals may be needed in some of the groups to establish more definitively some effects that are currently of borderline statistical significance.

The development of an autoradiographic method for the measurement of local rates of protein synthesis in brain by C. Smith and Y-L. Sun can now be considered to be complete. It is now fully operational and is being applied to a number of physiological and pharmacological states in which changes in protein synthesis and/or protein turnover might be expected. The critical final step in the development of the method measurement of protein synthesis was the development of an technique to assay the dilution of the precursor amino acid pool for protein synthesis by amino acids derived from protein degradation. This information is essential to determine local rates of protein synthesis in brain *in vivo*. Without correction for this dilution the protein synthesis method would measure only minimal possible rates of amino acid incorporation; actual rates would be greater. In the previous two years a method was developed to assay the dilution in the whole brain based on the steady state ratio of the whole brain tRNA-bound amino acid specific activity to the specific activity of the same amino acid in the plasma. In the absence of any dilution this ratio would be 1.0. In the presence of dilution the value of the ratio would be less than 1.0 and would decline with increasing degrees of dilution. The results of their application of this technique to normal rats showed that the flux of amino acids derived from protein breakdown is normally approximately 40-50% (i.e., dilution factor = 0.5-0.6) of the total amino acid flux into the precursor pool for protein synthesis, the remainder coming from the plasma. The technique that was used to determine the dilution factor in the brain as a whole is not, however, applicable to small, discrete structures in brain because the amounts of tRNA-bound amino acids in such small samples of tissue are too low to be measured. In the studies in whole brain, however, it was found that the dilution factor for the total tissue acid-soluble amino acid pool was smaller but almost linearly related to that for the true precursor tRNA-bound amino acid pool. This relationship was mathematically fitted, and the dilution factor can be calculated from the measured dilution factor for the acid-soluble amino acid pool, which is relatively large even in very small samples of brain tissue. Y-L. Sun punched out specific structures in normal brain, determined the dilution factors from measurements of the acid-soluble pool, and redetermined local rates of protein synthesis corrected for the dilution. This new technique, incidentally, provides a measure

of the rate of protein degradation in brain, a worthwhile measurement in its own right, particularly in view of the growing awareness that brain maturation and plasticity involves synaptic retraction as well as proliferation. A manuscript describing these studies has recently been published in the *Journal of Neurochemistry*.

In order to minimize the effects of the dilution of the precursor pool of amino acids, C. Smith, T. Dang, and Y-L. Sun attempted to overwhelm the dilution by using "flooding doses" of the labeled precursor amino acid. They found that such flooding with valine did, in fact, reduce the effects of dilution but did not completely eliminate it or the need to determine the dilution factors locally within the brain. This work was also published this year in the *Journal of Neurochemistry*.

Now that development of the method for determination of local rates of protein synthesis in brain, including correction for recycling of amino acids from protein breakdown, has been completed, a number of other studies, either in progress or newly initiated, can be completed. Smith and Sun are now applying the method to a variety of neurobiological states of interest: 1) effects of thiopental and ketamine anesthesia; 2) effects of acute functional activation, e.g., electrical stimulation of sciatic nerve on protein synthesis in all the components of the stimulated pathway; 3) time course of the effects of axotomy of the hypoglossal nerve on protein synthesis in the hypoglossal nucleus; 4) effects of normal aging; 5) effects of postnatal development; 6) effects of thyroid hormones on protein synthesis in normal adult brain and in the hypoglossal nucleus during regeneration of the axotomized hypoglossal nerve, etc. The results thus far indicate that there are indeed changes in both protein synthesis and protein degradation in some of these conditions, but the results are still too preliminary to allow definitive conclusions.

Significant progress was made in the new research project initiated last year on the possible role of nitric oxide (NO) in the coupling of local cerebral blood flow (lCBF) to local cerebral energy metabolism and/or functional activity. It has been known for at least 100 years that lCBF is adjusted to local tissue metabolic demand and functional activity, but the mechanisms of this regulation remain unknown. There is continuous controversy about whether the regulation is neurogenic or chemical. The most common belief is that the regulation is primarily through chemical factors. The cerebral vessels do have innervations of both sympathetic and parasympathetic origins, but there has been no convincing demonstration of any functional role for them, except possibly to adjust the range of arterial blood pressure over which autoregulation operates. On the other hand, the chemical consequences of increased energy metabolism, e.g., increased tissue pCO_2, decreased pO_2, and increased H^+ and the release of K^+, adenosine, adenine nucleotides, etc. into the extracellular space are all potent vasodilators, and they are tonically active so that when altered in the opposite direction by decreased energy metabolism, cerebral vasoconstriction occurs. Each of these chemical factors has been scrutinized in detail for years, and none of them has been proved adequate to explain all aspects of the regulation. That such regulation exists has been unequivocally proved primarily by the work of this Laboratory, which employed the methods that it developed to measure local cerebral blood flow and

glucose utilization in animals with quantitative autoradiography and in man with PET. There is no question that increased local functional activity is associated with accelerated local energy metabolism and that both are associated with increased local blood flow in man, and the converse is also true. The mechanisms underlying these associations are still obscure. Recent developments have suggested a new and unexpected candidate. Furchgott and others have identified an Endothelium-Derived Relaxing Factor (EDRF), now identified as nitric oxide (NO), that mediates the vasodilator effects of acetylcholine and a number of other vasodilator drugs. NO is synthesized in the vascular endothelium from arginine by a Ca^{++}/calmodulin-dependentmonooxygenase (NO synthase). The NO can diffuse out of the endothelium to the vascular smooth muscle where it activates guanylyl cyclase and causes a rise in cyclic GMP. NO synthase also exists in neuronal and glial elements and has been purified from the cerebellum. Conceivably the NO produced in the brain tissue can diffuse to the vascular smooth muscle as readily as the endothelium-produced NO. The NO is inactivated by free radicals, protected by superoxide dismutase, and normally has a half-life of about 1/2 minutes. NO, therefore, has many of the appropriate attributes of a mediator of cerebrovascular regulation. Because of its expertise in the methods for measuring lCBF and local cerebral glucose utilization ($lCMR_{glc}$), both of which it developed, and its long term interest and experience in problems of the relationships among local blood flow, energy metabolism, and functional activity in brain, this Section is uniquely suited to investigate the possible role of NO in the mechanisms of these relationships. Competitive inhibitors of the NO synthase, N^G-monomethyl-L-arginine (NMMA) and N^G-nitro-L-arginine methyl ester (NAME), have been used to block NO synthesis and determine if such blockade inhibits the cerebral circulatory response to the elevation of arterial pCO_2, known to increase cerebral blood flow, or to functional activation known to increase local cerebral energy metabolism. These studies have been carried out by C. Kennedy, K. Adachi, F. Wang (Guest Researcher), and S. Takahashi. The results demonstrated that there is normally some tonic vasodilator action of NO because the inhibitors produced dose-dependent decreases in cerebral blood flow despite correspondingly dose-dependent increases in arterial blood pressure, but the cerebral vasodilator effects of breathing 5% CO_2 were unaffected by the blockade of NO synthesis. Similarly, the increases in local blood flow in the components of the whisker-to-sensory cortex pathway produced by vibratory stimulation of the whiskers in the rat were unaffected by the inhibition of NO synthesis. It appears , therefore, that NO does not mediate the coupling of lCBF to local energy metabolism and/orfunctional activity. The results of these studies were reported recently at a symposium on the Pathophysiology of Cerebral Ischemia in Marburg, Germany, and a manuscript on this report is in press.

B. Driscoll has made significant progress in his studies of development of neurons and astroglia cultured in chemically defined media. The neurons, which are derived from the embryonic mesencephalon of fetal rat brain, are destined to become dopaminergic, and he evaluates their development morphologically and functionally by assay of their dopamine-reuptake system, a measure of their neurite development and maturation. Early in the course of these studies he found that NMDA receptors play a role in the survival of these dopaminergic neurons in culture. After a certain age in culture, presumably when the NMDA receptors have developed, these neurons acquire a sensitivity to glutamate in the

culture medium. Glutamate in the medium in concentrations as low as 10 μM causes cellular damage and death. Replacing the culture medium with fresh medium also damages or kills the cells, but agents that block the NMDA receptors, e.g., MK-801 or 2-amino-5-phosphono-valeric acid (AVP), completely protect the cells against both glutamate and medium change. Driscoll has found that many commercial media used for cell cultures contain glutamine which are either contaminated with or can be converted to toxic levels of glutamate. These studies were published previously in the *Journal of Neurochemistry*.

During the past year Driscoll has found that only a very low level of glutamate (e.g., μM range) is needed to initiate the cellular damage but that the events mediated via the NMDA receptor are completed within a few minutes after the change of medium. It is during the next 3-6 hours that the cells are damaged by mechanisms that are still unknown. Over the subsequent 12 hours the glutamate level in the extracellular medium rises by several hundred nmols/ml. This glutamate appears to be produced extracellularly because it is also appears in medium which has been removed and cultured again in the absence of cells. A reasonable hypothesis to explain this surprising effect is that neurons damaged in the first few minutes after the medium change leak glutaminase into the culture medium, and the glutaminase converts the glutamine in the medium to glutamate. If similar mechanisms were to operate *in vivo*, injured neurons in a damaged region of brain could leak glutaminase and thus generate large quantities of glutamate from the abundant glutamine normally present in the extracellular space. This glutamate would then lead to further damage of healthy neurons in the region, and a cascade of ever widening cell damage and death would then ensue. This hypothesis is currently under study. A manuscript reporting these more recent findings is almost ready for submission to the *Journal of Neurochemistry*.

Driscoll has also been using his cell cultures in comparative studies of the carbohydrate metabolism of neurons and astroglia. The oxidative metabolism of glucose is the almost exclusive source of cellular energy in the nervous system. Work of this Section during the past few years established unequivocally that glucose utilization is enhanced with increased functional activity in nervous tissue and that the increases in metabolism occur almost entirely in the neuropil and little if at all in perikarya. The neuropil consists of neuronal axonal terminals and dendrites and is rich in synapses, but there are also astroglial processes surrounding the synapses. It is difficult, if not impossible, to determine if the increased energy metabolism is in the neuronal elements, in the astroglia, or in both, and it is hoped to approach this question by studying the glucose metabolism of neurons and astroglia cultured separately *in vitro*. The deoxyglucose method for measuring local glucose utilization can readily be adapted for use *in vitro*. In the initial experiments in this project Driscoll has examined the steady state intracellular/extracellular distribution ratios (i.e., distribution spaces) of both 2-[^{14}C]DG and glucose in neuronal and astroglial cell cultures over a wide range of glucose concentrations in the medium mimicking hypoglycemia, normoglycemia, and hyperglycemia, about which much is already known *in vivo*. The results show that both cell types handle DG similarly with essentially equal distribution spaces at all concentrations of glucose in the medium. The results with glucose are more complicated,

particularly with the hypoglycemic levels of glucose in the medium, probably because of a residual pool of glucose equivalents stored as glycogen in the glia. The next stage is to compare the kinetics of hexose transport in the neurons and glia and to relate them, if possible, to the results of examination of the glucose transporters in the two types of cells.

E. Kaufman and B. Driscoll have continued their studies of the metabolic interdependence of glia and neurons in brain. They have been studying the regulation of the anaplerotic reaction, (CO_2 + pyruvate → oxalacetate), which is catalyzed by an exclusively glial enzyme, pyruvate carboxylase. They found that $^{14}CO_2$ fixation by this reaction in astroglia is stimulated by increases in extracellular K^+ concentration in the range that occur *in vivo* with normal neuronal functional and electrical activities. It appears, however, to be unaffected by agents that depolarize neurons, such as veratridine, when the assay is conducted on astroglia in the absence of neurons. When neurons are absent from the medium, there is an accumulation of ^{14}C-labeled organic and amino acids, somewhat in the cell bodies but mainly in the medium; presumably these are metabolic products of the tricarboxylic acid pathway. When neurons are included in the incubation mixture, the accumulation of these products in the cell bodies is the same but there is little accumulation in the medium. These results provide evidence of the metabolic interdependence between the neurons and astroglia. This work was recently published in the *Journal of Neurochemistry*. Work is continuing on the identification of the labeled products of the $^{14}CO_2$ fixation that are passed from the astroglia to the neurons.

E. Kaufman and T. Nelson have continued their studies of the biosynthesis, degradation, and physiological functions of γ-hydroxybutyrate (GHB). In large pharmacological doses of 200-400 mg/kg in rats this compound produces a reversible trance-like state characterized by an almost isoelectric EEG and a profound depression of glucose utilization throughout the brain. The compound is, however, normally present in brain in small amounts (e.g., 1-4 μM), but its biosynthesis, degradative pathway, and normal physiological role remain largely unknown. Previous work on this project identified two degradative enzymes, a cytosolic $NADP^+$-dependent GHB dehydrogenase that oxidized GHB to succinic semialdehyde and a mitochondrial hydroxyacid-oxoacid transhydrogenase that converts the GHB to succinic semialdehyde and the co-substrate α-ketoglutarate to α-hydroxyglutarate by transhydrogenation. The relative quantitative importance of the two pathways is under further study. Work on the effects of GHB during the last year has been carried out *in vitro* on primary neuronal and astroglial cell cultures provided by B. Driscoll and also on homogenates of hypothalamus and hippocampus. The results indicate that GHB in the range of concentrations between 1 and 100 μM inhibits cAMP formation in the cultured neurons and in the hippocampal and hypothalamic homogenates, but not in the astroglia. This work is being continued and extended to the effects of GHB on Ca^{++} transport; preliminary results suggest that GHB may also inhibit Ca^{++} uptake by unstimulated neurons in culture.

Section on Clinical Brain Imaging

Louis Sokoloff, Acting Chief
(Report prepared by Alan Zametkin)

The objectives of the Section on Clinical Brain Imaging (SCBI) are the development of new methods to study normal and abnormal physiology in the brain by positron emission tomography (PET) and the application of these methods to the study of neuropsychiatric disorders. The two major areas of effort during the past year were the Attention-Deficit Hyperactivity Disorder (ADHD) in adult and teenage subjects and the development of the [^{18}F]DOPA PET method and the evolution of its application from non-human primates to human subjects.

The studies on ADHD focused on three areas: 1) Examination of the regional cerebral metabolic effects of acute and chronic administration of the CNS stimulants, dextroamphetamine and methylphenidate, has been completed. The results of the studies of the acute effects of these drugs have been submitted for publication, and the data on the chronic effects of the drug are currently being analyzed. Both stimulants appear to have modest effects on metabolism in the brain but produce positive behavioral improvement. 2) By use of a new method developed by A. Zametkin, the Section has been able to apply the [^{18}F]FDG method with PET scanning on minors with minimal radiation exposure. A manuscript on the description and initial application of this method is in press in the *Archives of General Psychiatry*. The method is now being applied to normal teenagers and those with ADHD. In addition, teenage schizophrenics are being scanned in collaboration with the Child Psychiatry Branch, NIMH. 3) ADHD is highly associated with Generalized Resistance to Thyroid Hormone (GRTH), a disorder with a defined genetic defect. Studies in collaboration with the Molecular and Cellular Endocrinology Branch, NIDDK, have revealed that ADHD is highly associated with GRTH in the NIDDK study group and that these patients have impaired attention and altered glucose metabolism, as measured with [^{18}F]FDG and PET, in brain areas related to attentional processes.

D. Doudet and R. Cohen have extended their [^{18}F]fluoro-Dopa PET method to human subjects from earlier work in monkeys. By late infusion of unlabeled amino acids, the visualization of brain regions containing dopaminergic neurons is greatly improved. In collaboration with the Neuropsychiatry Branch, NIMH, both normal control subjects and schizophrenic patients are currently being studied to understand better the role of dopaminergic systems in brain function. D. Doudet is also using the [^{18}F]fluoro-DOPA method to assess dopaminergic function in cocaine-treated rhesus monkeys.

A. Zametkin is also collaborating with T. Sunderland of the Unit on Geriatric Psychopharmacology, NIMH, in the use of a double [^{18F}]FDG technique to examine the effects of scopolamine on regional brain metabolism in elderly adults, in order to

understand better the memory impairment associated with Alzheimer's Disease. Initial results suggest altered metabolism in brain regions related to cholinergic functions.

Collaborative studies with Dr. Andreason of the Laboratory of Clinical Studies, NIAAA, in which PET is used to determine the neurobiological correlates of chlorophenylpiperazine (mCPP) action in early-onset alcoholics, are continuing.

The Section continues to train and provide assistance to other research groups who do or wish to employ PET-imaging techniques and associated data analysis in their research programs. During the past year, Dr. Schmidt under the direction of Dr. Potter of the Clinical Pharmacology Section have initiated a study to apply the double [^{18}F]FDG technique to evaluate the effects of Idazoxan in depressed patients and normal controls.

The Section plans to use the [^{18}F]fluoro-DOPA method, developed by members of this Section, to define better the involvement of dopaminergic system in ADHD. The twin goals of the Section, method development and application to neuropsychiatric disorders, may now be realized in a single research project.

Annual Report of the
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Giulio L. Cantoni, M.D., Chief

As in earlier years the biochemistry and biology of S-adenosylmethionine (AdoMet) and its role in transmethylation reactions continued to be the dominant interest of the LGCB. The biochemical versatility of AdoMet is unique in biology; because of its chemical structure AdoMet serves as a methyl donor to a very large and quite diverse group of methyl-acceptor compounds, as an adenosine donor, as a butylamine donor and after decarboxylation, as a propylamine donor. In addition AdoMet serves as an allosteric effector in several enzyme systems.

S-adenosylmethionine was discovered forty years ago and the importance of this molecule in many diverse biological systems continues to command center stage. The projects that are being pursued in the Lab are a reflection of different aspects of the biochemistry of AdoMet and comprise both biochemical and biological aspects of the general theme. The division of our research effort in separate projects is therefore somewhat artificial.

The contributions of each member of our small group are in all respect, equivalent at the conceptual level; at the technological level each member of the group contributes specialized know how that is utilized for the benefit of the various projects without jealousy.

AdoMet dependent methyl-transfer reactions are involved in a very large number of reactions in all phyla: quantitatively, it has been established that more than 5% of all the enzymes characterized to date catalyze AdoMet-dependent methyl-transfer reactions; qualitatively, methyl-transfer reactions play a pivotal role in cellular differentiation, in chemotaxis, as well as other forms of signal transduction, and in the synthesis of a great number of key metabolites.

One of the products of methyl transfer reactions is S-adenosylhomocysteine, AdoHcy, a competitive inhibitor of methyl transfer reactions. The Ki of different methylases for AdoHcy and the Km for AdoMet varies over a wide range. This indicates that at any given intracellular concentration of AdoMet and AdoHcy the relative activity of different methylases would be different and suggested to us that the ratio of the intracellular concentration of AdoMet and AdoHcy might be a key factor in the mechanism or mechanisms that control the biological utilization of AdoMet.

The hypothesis linking regulation of methyltransferases to the ratio of the intracellular concentration of AdoMet and AdoHcy led to the formulation of a research strategy having two principal objectives: 1) the study of the enzymology, molecular properties and eventually regulation of enzymes that synthesize or utilize S-adenosylmethionine and S-adenosylhomocysteine, and 2) the study <u>in vivo</u> of biological consequences of the modulation of the AdoMet/AdoHcy ratio; this can be best achieved either by the inactivation or the inhibition of AdoHcyase or by use of 3-deazaadenosine, an analog of adenosine that as we discovered a few years ago can be utilized by the enzyme to synthesize congeners of AdoHcy having somewhat different inhibitory specificity.

The enzymology of AdoMet and AdoHcy

In every cell the synthesis of AdoMet is catalyzed by methionine-adenosyl-transferase (MAT) an enzyme discovered by Cantoni. MAT catalyzes a reaction without analogy in biochemistry as it involves the utilization of the energy of the pyrophosphate bonds of ATP for the generation of a novel energy rich sulfonium bond. In several species MAT is represented by a family of isozymes that differ in their kinetic and molecular properties. MATs from several different sources have been cloned and sequenced and the comparison of their primary amino acid sequences reveals an extraordinary degree of homology. The biological significance of the presence of several MAT isozymes in the same organism, and even in the same tissue, is not clear and deserves further study.

While the crystal structure of the enzyme has not yet been determined, comparison with other enzymes that utilize ATP has revealed to us a hitherto unrecognized degree of homology between an amino acid sequence identified as the ATP binding domain of adenylate kinases and a region in MAT enzyme. We plan to extend this observation by means of a variety of approaches (chemical labeling, model building and site-directed mutagenesis).

In the last few months we have made considerable progress in analyzing the comparative metabolism of AdoHcy in different phyla, and the results are of great interest. An individual organism metabolizes AdoHcy by only one of three different pathways: In several species of bacteria, in fungi and in all eukaryotes AdoHcy is metabolized through a single pathway catalyzed by AdoHcyase, an enzyme discovered many years ago in this lab and extensively studied here. In enterobacteria and in most species of mycoplasma AdoHcy is cleaved irreversibly to adenine and ribosylhomocysteine by a specific nucleosidase; ribosylhomocysteine is metabolized to homocysteine and a poorly characterized ribose compound. In archibacteria AdoHcy is rapidly deaminated to inosylhomocysteine. What is the biological significance of these different pathways?

In vertebrates AdoHcyase fulfills an indispensable metabolic requirement since cleavage of AdoHcy by AdoHcyase generates homocysteine, a compound that serves as the sole precursor of cysteine, a key amino acid that is not an essential dietary requirement.

By contrast AdoHcyase is not needed by Enterobacteria since they are able to synthesize cysteine from hydrogen sulfide and serine; however these species may require the adenine moiety of AdoHcy that is generated by the specific nucleosidase. Little is known about the metabolic requirements of archibacteria and we hope to explore this area of metabolism in the near future.
The discovery that the metabolism of AdoHcy in mycoplasma and in enterobacteria is similar has important implications as to the evolutionary origin of mycoplasma.

Considerable effort has been devoted towards elucidation of the enzymology and molecular biology of AdoHcyase and much progress has been achieved. In eukaryotes AdoHcyase may be classified as a housekeeping or as a tissue specific enzyme since its activity is present in every tissue but varies more than 100 fold in different tissues. We have established that regulatory control must occur at the level of transcription, since in different tissues the amount of mRNA is correlated with the level of expression. Elucidation of the genomic

organization of AdoHcyase is required before we can understand the mechanism of transcriptional control. The genomic organization appears to be fairly complex and in the rat the AdoHcyase gene covers approximately 90kb.

The enzymes from rat liver, Dyctiostelium discoideum and Rhodobacter capsulatus have been cloned and sequenced in our laboratory and the human enzyme has also been cloned using the rat liver cDNA as a probe. The sequence of AdoHcyase from parsley leaves, Caenorhabditis elegans and Leishmania donovani have been obtained and show a very high degree of homology with the amino acid sequence of the rat liver enzyme. In fact, to our knowledge, the conservation between the human and Rhodobacter sequences is the highest so far reported between enzymes from species that are separated in evolutionary time by more than two billion years. One feature of the enzymes from Rhodobacter and parsley is of particular interest, namely the presence of 36 amino acids approximately one third of the distance from the amino terminal, that is not found in the rat, human, D. discoideum or C. elegans enzymes. Rhodobacter mutants lacking AdoHcyase have been obtained and are the first example of a null mutation in AdoHcyase, We have shown that these mutants will grown normally if supplied with methionine but that they are unable to synthesize their photosynthetic apparatus. As noted earlier several years ago we formulated as a working hypothesis the theory that modulation of the intracellular ratio of AdoMet to AdoHcy would play a key role in the regulation of biological methylation reactions, and that AdoHcyase would play a pivotal role in this connection. The fact that in these mutants the intracellular accumulation of AdoHcy inhibits the synthesis of bacteriochlorophyll, but does not inhibit growth provides compelling and direct evidence that the hypothesis we formulated, and have tried to verify experimentally for a long time, is in fact correct.

Analysis of the amino acid sequence of AdoHcyases revealed the presence of a sequence of 31 amino acids with the characteristics of the dinucleotide binding domain found in several unrelated dehydrogenases. By site directed mutagenesis we were ableto show that substitution of critical amino acids in this domain leads to loss of NAD binding and of catalytic activity. These results establish experimentally for the first time the validity of the assignment of NAD binding in AdoHcyase to the so called nucleotide cleft.

Investigation of the inactivation of the enzyme by FSBA (fluorosulfonylbenzoyladenosine) led to the identification of cysteine 78 as a highly reactive residue probably involved in the determination of the tertiary structure of the enzyme.

Physiological Correlates of Biological Methylation

We have reported a few years ago that macrophage chemotaxis can be inhibited by ethionine or 3-deazaadenosine, two compounds that are known to modulate methylation reactions in vivo by altering the AdoMet/AdoHcy ratio. Macrophage chemotaxis may be viewed as a model for receptor mediated signal transduction mechanism. Analysis of the inhibition of chemotaxis by compounds that affect methylation reactions allowed us to rule out the role of phospholipid methylation, arachidonate release and methylation of lysine or arginine residue in proteins in this phenomenon. In an extension of this line of work, we found that the incubation of macrophages with pertussis toxin is correlated with the inhibition of chemotaxis and with the ADP-ribosylation of a 41,000 MW guanine nucleotide binding protein. Later we reported that inhibition of chemotaxis by

cholera toxin is associated with ADP-ribosylation of a different guanine nucleotide binding protein. Guanine nucleotide binding proteins are a family of membrane heterotrimers composed of common βγ-subunits and different ADP-ribosylatable α-subunits. In the macrophage cell line we have been studying we have established that a specific guanine nucleotide binding protein, Gi-2 is a major substrate for ADP-ribosylation by both cholera and pertussis toxin. A connection between these results and our earlier finding that chemotaxis is inhibited by methylation inhibitors was provided by the discovery that membrane proteins from macrophages are carboxylmethylated by a membrane bound methyltransferase in a reaction that is greatly stimulated by guanine nucleotides. Protein carboxylmethylation is unique among methylation reaction in that while biochemically irreversible, like all reaction involving transfer of the methyl group of AdoMet, it is physiologically reversible since methyl esters can undergo enzymatic or chemical hydrolysis. A guanine nucleotide dependent carboxylmethylation system provides therefore, an attractive system for regulating the function of guanine nucleotide binding proteins in signal transduction. Recently it has been established that a common carboxylterminal amino acid sequence is present in a number of membrane proteins. This sequence, Cys-Aaa-Aaa-Xaa, is modified by a series of reactions that include farnesylation and carboxylmethylation of the cysteine residue after proteolytic cleavage of the Cys-Aaa bond. A cytoplasmic protein that exhibits GTP-dependent carboxylmethylation has been identified in the laboratory as G25K and purified to homogeneity from brain homogenates. The methyltransferase responsible for methylation of G25K is membrane bound. The role of GTP in the carboxylmethylation reaction is to increase the affinity of G25K for the methyltransferase. The soluble form of G25K forms a heterodimer with a protein of 28 kDa and the association of G25K with the 28kDa protein inhibited the methylation of this G protein. These results will facilitate our understanding of the relationship between carboxylmethylation and intracellular localization of this class of GTP-binding proteins.

Chemotaxis in macrophages as in bacteria now appears to involve participation of analogous albeit different carboxylmethylation reactions. It will be of interest to explore further the broad analogies between these two systems of signal transduction and investigate the possible reversibility of the methylation of G-protein by carboxylmethyl esterases.

In vertebrates an inverse relationship between gene expression and DNA methylation is now well established but its mechanism is incompletely understood. Housekeeping genes, which are expressed in every cell are characterized by relatively high densities of unmethylated CpG islands in the promoter region; whenever these CpG residues become methylated the genes become transcriptionally inert. By contrast, in tissue specific genes that are normally repressed, CpG sequences are methylated but become hypomethylated where, or when, the specific genes are expressed. It is noteworthy that the effect of promoter methylation on gene repression can be best demonstrated <u>in vivo</u>. We have investigated two aspects of this multifaceted problem: a) the biochemical mechanism underlying conversion of mCpG residues to CpG residues, and b) the mechanism linking promoter methylation and inhibition of gene expression.

Conversion of mCpG to CpG by removal of the methyl group from methyl cytidine is biochemically impossible as this reaction would involve cleavage of a C-C bond; in order to account for the loss of mCpG during gene expression two different models have been proposed: i) we and others have shown that hypomethylation

occurring concomitantly with cell duplication can be explained by inhibition of maintenance methylase through two cycles of DNA replication ii) in the absence of DNA duplication hypomethylation involves replacement of a large fraction of mC in mCpG sequences with cytidine as first demonstrated by Cantoni, Razin and collaborators.

The mechanism by which the presence of methyl groups at the promoter region inhibit gene activity has been pursued by use of engineered constructs in transient transfections. The most effective suppression was observed when methylation was in the preinitiation domain. Our recent results support an earlier suggestion that a mediator protein is involved in the mechanism of promoter inhibition.

Biological methylation and human disease

The well established therapeutic efficacy of Adomet in depressive disorders led Cantoni and his collaborators to advance the hypothesis that at least some of these conditions may be related to deficiency of AdoMet and/or methylated intermediates in the brain of affected individuals. Brain MAT differs from the two liver isozymes in some key kinetic properties and it may be postulated that a defect in this enzyme might play a role in the etiology of depressive disorders. Validation of this hypothesis requires elucidation of the structure of the brain specific MAT isozyme and comparison with other MAT isozymes; a project with this objective has been started in the last few months in collaboration with Ed Ginns.

As should be clear from the preceding discussion in humans and other vertebrates the hydrolysis of AdoHcy is the only source of homocysteine. Homocysteine lies at an important metabolic branch point: it may be utilized in the sulfur conservation pathway and methylated to form methionine or it may be converted to cystathionine and enter the transulfuration pathway that converts the sulfur atom of methionine to the sulfur atom of cysteine. The transulfuration pathway is the main catabolic route of methionine and explains why cysteine is not an essential amino acid in humans.

In healthy adults, homocysteine is metabolized effectively through these two pathways and the level of homocysteine in the blood is quite low. Decreased rates of metabolism through either of these two pathways leads to the accumulation of homocysteine resulting in hyperhomocysteinemia and homocysteinuria. Harvey Mudd, for many years a valued member of the LGCB, was the first to recognize the variety of metabolic lesions that cause homocysteinuria and has made major and fundamental contributions to the pathophysiology of homocysteine metabolism. Since his retirement Harvey Mudd has continued to work with us as a guest worker. Dr. Mudd has recently reviewed a series of clinical studies that support and extend the earlier conclusion of the Wilcken's, that mild homocysteinemia is a risk factor for premature ischemic heart disease. In most cases the risk associated with hyperhomocysteinemia is independent of other recognized risk factors such as hyperlipidemia, hypertension, diabetes and smoking. These conventional risk factors account for only 50 % of the overall incidence of atherosclerotic disease. In fact there is reason to believe that hyperhomocysteinemia is better as a predictor for atherosclerotic vascular disease that hypercholesterolemia.

Further work is needed to define the absolute risk associated with hyperhomocysteinemia due to heterozygosity for cystathionine β-synthase deficiency but it is important to point out that therapeutic interventions with pyridoxine, folate and or betaine, alone or in combination, may greatly reduce the risk of vascular disease in the population of the Unites States. A prospective long range study along these lines should be considered.

Miscellaneous: Dr. Cantoni, with the collaboration of Drs. S. Kety, N. Siliprandi and V. Andreoli, was primarily responsible for the organization and the management of an international workshop on Genetics and Gene Expression in Mental Disease. The workshop was held in Venice, Italy from October 28, 1991 to October 31, 1991 under the sponsorship of the Istituto Veneto di Scienze, Lettere ed Arti, with the participation of about 25 invited lecturers from the United States, England, Sweden, Israel and Italy. The proceedings of the workshop will be published in a special issue of the Journal of Psychiatric Research, and are currently in press.

Annual Report of the Laboratory of Molecular Biology
National Institute of Mental Health
October 1, 1991 - September 30, 1992
Howard A. Nash, Ph.D., Chief

Introduction

Research in the Laboratory of Molecular Biology addresses basic questions about the mechanisms of biological processes. The three independent Sections of the Laboratory operate independently but provide intellectual and technical support for each other. In the past year, work in the Sections has made advances in understanding protein translocation across membranes, opiate receptor structure, the targets of general anesthetics, cellular responses to HIV infection, and the rearrangement of genetic material.

Section on Molecular Genetics

Howard A. Nash, Ph.D., Chief

The Process of Lysogeny

This project explores the way a programmed rearrangement of DNA is achieved in nature. We focus on the reaction that integrates the DNA of a bacterial virus, phage lambda, into a specific locus on the genome of its bacterial host, E. coli. We want to understand how interactions between specific proteins and their DNA targets accomplish a precise and efficient joining of two pieces of DNA.

In the lambda system genetic exchange between virus and host involves breakage of parental DNAs and subsequent rejoining of the broken ends to each other. In biological systems, breakage of DNA usually involves attack of the phosophodiester backbone by a nucleophile. Int protein can use a nucleophile from one of its tyrosine residues to attack the phosphodiester backbone of DNA and simultaneously create a covalent enzyme-DNA bond. There is much evidence that such cleavage is involved in the initial step of recombination at the bacterial recombination locus, attB. However, it is not clear which nucleophile attacks the corresponding strand of the attP partner. While a second protomer of Int could provide this nucleophile, it is equally plausible to imagine that the nucleophile comes from the DNA fragment of that attB that is created by the first attack. We have distinguished between these alternatives by manipulating the enzyme and its DNA substrates. Our data lead us to conclude that there is symmetry in the mechanism of integrative recombination; like attB, attP is attacked by an enzymic nucleophile.

The active form of the viral recombination locus, attP, is a compact nucleoprotein structure that comprises a long segment of DNA decorated with multiple copies of the recombinase protein, Int. Accessory proteins that bend DNA are needed to help assemble this compact structure. E. coli encodes several families of proteins that deform DNA. In the past we have identified one such binding protein, E. coli Integration Host Factor (IHF), that clearly contributes to this process. Strong homology between their amino acid sequences indicates that IHF protein is structurally related to the HU proteins, a family that is widely distributed in procaryotes, mitochondria and chloropalasts. Like IHF, members of the HU family bind and deform DNA, but unlike IHF, HU proteins bind to DNA and with low affinity and with little or no sequence specificity. Previous work from our laboratory has shown that HU cannot replace IHF for integrative recombination but we now have demonstrated that excisive recombination, the site-specific deletion of the integrated viral chromosome, can be promoted by purified HU protein. We believe that IHF enhances both integrative and excisive recombination by deforming DNA so as to facilitate construction of precise and compact nucleoprotein arrays. To test whether HU substitutes for IHF in this function or whether it stimulates excisive recombination by a

different mechanism, we examined the structure of one of the excisive recombination loci, attL. Our data indicates that HU contributes to the formation of a complex in which Int protein bridges two non-adjacent sites in attL. HU is trapped in this complex and thereby gains the site-specificity which it lacks on its own. The failure of HU to replace IHF in integrative recombination suggests that alternate structures are formed and that some of these must be too weak to successfully trap HU.

In unicellular organisms, the capacity to break and rejoin specific DNA sites has been exploited for a variety of biological purposes. These include control of gene expression, manipulation of gene dosage, and faithful segregation of chromosomes during mitosis. In multicellular organisms, the generation of diversity in the immune system involves site-specific recombination but this appears to use a substantially different mechanism from that found in unicellular organisms. To inquire about potential novel biological roles for site-specific recombination, we have investigated whether homologs of site-specific recombinases of the Int family are more widely disseminated than is currently appreciated. We designed degenerate sets of oligonucleotides that conform to the conserved portion of six recombinases and of the Int family that are found in episomal elements of various yeast species and used them as PCR probes. To identify the genomic sequences highlighted by our probes, we isolated individual clones from a genomic library that produced the desired signal. We focused on the genome of S. pombe because its small genome simplified the search, it is only distantly related to the yeast species known to have an Int-like recombinase, and its abundance of introns and their mechanism of splicing suggest that this organism is more closely related to higher eucaryotes than are other yeasts. The DNA sequence of isolated clones showed that the match to our primers was accidental and did not arise from an open reading frame that encoded a gene with significant homology to the recombinases. However, the DNA sequence from the region did reveal previously unidentified open reading frames. Comparison with genes in the database suggests that these are the S. pombe equivalents of the ARG4, ILV5 and RPB6 genes of S. cerevisiae, genes involved in arginine isoleucine and messenger RNA biosynthesis, respectively.

Genetic Neurobiology of Drosophila

The nervous system of Drosophila contains tens of thousands of nerve cells organized into diverse structures that control an impressive array of complex behaviors. This project seeks to apply genetic techniques to an analysis of the action of general anesthetics in Drosophila. In the long term, we want to use mutations to identify the critical target(s) whose alteration produces the anesthetic state and we want to use general anesthetics as a tool to find genes that govern neural function in the fruit fly.

In higher organisms, it has been repeatedly observed that there is a remarkable correlation between the dose of an agent required to produce anesthesia and the hydrophobicity of that agent, as measured by its solubility in olive oil. This correlation is not only valuable in predicting the potency of new agents, it provides the lone insight to the nature of the anesthetic target, i.e. it must be hydrophobic. We have now measured in Drosophila dose-response curves for nine volatile anesthetic liquids. We find that for each anesthetic, ED50 asymptotes to a fixed value in the course of one to three hours, thus establishing a time-independent measure of potency. We have also used gas chromatography and glass chambers to measure the way these agents partition between air and olive oil. We find that, just as for higher organisms, there is an excellent correlation in Drosophila between hydrophobicity and potency. Moreover, the absolute concentration of volatile agents required to produce anesthetic effects is quite comparable to that seen with mammals. These results strongly support the contention that the anesthetic target has been conserved throughout evolution.

We have previously shown that n-alkanes of chain length less than ten can act as anesthetics in Drosophila. We have now measured the olive oil/gas partition coefficients for these alkanes and find that there is a significant disparity between the observed potency and that predicted by their oil solubility; the alkanes are less potent than expected and the discrepancy increases with increasing chain length. We have also shown that flies recover from alkanes anesthesia as rapidly as from conventional anesthetics. This argues against artifacts introduced by rapid metabolism of alkanes. We conclude that alkanes are an unusual anesthetic, one whose target is not well modeled by olive oil. The same conclusion has been independently reached by Eger and colleagues in a recent study of alkane anesthesia in rats. These findings confirm our contention that the anesthetic target has been conserved.

Our previously isolated mutants that change the response of Drosophila to halothane should provide clues about the components of the nervous system that are affected by anesthetics. One way to ask about the nature of the changed component is to determine its anatomical sphere of action, i.e. which tissue of Drosophila needs to be mutant in order to produce a mutant phenotype. We have begun this search by determining the role of the head. We cut the connections between the head and the body, allowed the flies to stabilize overnight, and tested them for their responsiveness to anesthesia. The results leads us to conclude that one mutation affects a component that resides in the head or depends upon a signal from the head while the other alleles affect autonomous neural elements in the body. Thus, the decapitation assay has distinguished at least two anatomical sites of gene action.

Using a new assay that measures the capacity to respond to a noxious stimulis, we have determined the response of wild-type and mutant flies to several anesthetics other than halothane. We found that each of the three loci tested show a pattern of response that is different from wild-type flies and different from each other. On two grounds our data indicate that the mutations alter pharmacodynamics and not just pharmacokinetic aspects of anesthesia. First, an alteration in ED50 is seen in the study state, so models that invoke altered rates of passive entry and/or distribution of anesthetics are eliminated. Second, in several cases a mutant line that is altered in response to an anesthetic in the new assay is not altered in the a second assay. This eliminates models that postulate differences in overall metabolism of the anesthetic. Because the new data indicate a pharmacodynamic modification in a fundamental anesthetic trait, they complete the disproof of the classical view of anesthesia which suggests that all volatile anesthetics have identical effects at the anesthetic target.

Section on Regulatory Proteins
Werner Klee, Chief

The Biochemical Basis of Peptide Receptor Activity

Cell surface receptors are protein components of membranes which receive chemical signals from the environment and transmit this information to the interior. Many of these cell surface proteins, share strong structural and functional similarities with one another. In particular, a large group of receptors, which mediate the actions of a number of hormones and neurotransmitters, are coupled to the activation or inhibition of intracellular enzymes by GTP–binding regulatory proteins (G proteins). The activated G proteins serve as transmitters of information to the interior of cells. The neuroblastoma x glioma hybrid cell line, NG108-15, richly endowed with opiate receptors, is a particularly attractive experimental system since it expresses only one of the many opiate receptors found in brain. In the past year Klee and colleagues have focused on the isolation and sequencing of peptide fragments of opiate receptors purified from NG108-15 cells by procedures worked out in previous studies. The new work have required them to develop methods for increasing the yields of preparative procedures, preparing fragments of the purified protein by proteolytic digestion, and purifying these

fragments. They have determined the amino acid sequences of many fragments after their separation by micro-bore HPLC columns and have used this sequence information to prepare oligonucleotide probes with which they plan to isolate cDNA clones coding for the opiate receptor from an NG108-15 library.

By the combined use of specific antibodies and synthetic peptides Klee and colleagues had previously characterized a receptor domain that activates G-proteins, and showed that no other intracellular domain fully shares these properties. In a new initiative they have expressed a rat alpha2-adrenergic receptor in CHO and COS cells and have characterized the pharmacological efficacy of various ligands in this receptor system. Experiments are underway which will prepare mutants of these receptors in those regions of the transmembrane helices that are predicted to control the efficacy of adrenergic agonists. Their studies have already shown that receptor coupling is fairly inefficient in these cell lines and have prompted them to engineer COS cells that overexpress either Go, or Gi-2. These cells will be important tools for studying the properties of mutant receptors after transient expression, and may also provide useful new approachs to the expression cloning of opiate receptors should current efforts not meet with success. In conjunction with these experiments, studies aimed at improving the methods for predicting the three dimensional structures of proteins based upon amino acid sequence information are also being carried out. Such methods will be important tools for interpreting the results of cloning studies since knowledge of amino acid sequence will not by itself provide great insight into the mechanisms involved in receptor action.

Section on Biophysical Chemistry
David M. Neville, Jr., Chief

The Section on Biophysical Chemistry is concerned with the basic aspects of cell membrane biochemistry. Areas of active interest include receptor-mediated protein transport, protein translocation across membranes, membrane fusion events and signal transduction pathways. Knowledge derived from these basic processes are applied to the study of early steps in enveloped viral infections, the function of the HIV regulatory protein nef, and the rational construction of drug targeting schemes. Foremost of these is the development of anti-T cell immunotoxins to achieve *in vivo* T cell ablation for the treatment of leukemia/lymphoma, autoimmune diseases and graft-versus-host diseases.

Early Steps in Enveloped Viral Infections

Studies conducted with Drs. Akeson and Rigaut have shown that an early step in vesicular stomatitis virus (VSV) infection is blocked when the plasma membrane potential is reduced. This early step is just distal to viral endocytosis. If viral endocytosis is limited to a 0-5 minute pulse, inhibition is obtained by collapsing the membrane potential between 5 and 60 minutes after entry and results in an 8 fold diminution of viral particle output between 3-4 hours post infection. This effect does not appear to be mediated by changes in either intercellular calcium or pH.

The precise step affected is currently being analyzed. Viral membrane/plasma membrane fusion does not appear to be involved as judged by fluorescence dequenching studies using VSV loaded with the dye R18. Membrane translocation of VSV virons and subsequent solubilization of the VSV matrix protein is also not significantly inhibited as judged by anti-VSV matrix protein staining of infected cells blocked in their ability to synthesize new viral proteins. It would appear then that potential sensitive step is distal to translocation and matrix protein uncoating, yet occurring at a time which precedes the peak activity of primary VSV transcription (2 hours post infection). A working hypothesis is that an unknown obligatory processing step preceding

primary transcription is sensitive to the absence of a membrane potential. How this putative cytosolic processing step senses the membrane potential is an interesting unanswered question.

HIV Nef Function

Infection of humans by HIV and of certain primates by SIV (simian immunodeficiency virus) leads to an immunodeficiency (AIDS) with secondary, opportunistic disease and death. Early in the infective process these retroviruses express regulatory proteins, with the Nef transcript representing nearly 80% of total viral mRNA. Recent work by Desrosiers and colleagues has shown that *in vivo* SIV infectivity is achieved in the absence of Nef expression, but that there is an absolute requirement for Nef for the development of persistent high viral titers and for the development of immunodeficiency. On the other hand Nef has no apparent effect on SIV of HIV infection of cells in culture. The essential *in vivo* activity of the Nef protein in the development of AIDS has not been defined.

In order to define the biochemical activity of Nef Dr. Marsh's group set out to express Nef in murine T cell lines. This has been achieved by transduction with a retroviral expression vector. They chose to use murine T cells since the biochemical pathways for human and murine T cell function are highly homologous, and since the resources for studying murine T cell function far overshadow the possibilities of human studies. Nef expression was found to down-modulate the surface expression of CD4 and CD8ab, the two molecules known to interact with the T cell specific tyrosine kinase, $p56^{lck}$. Additionally, they have found that Nef expression in a murine T cell hybridoma sensitizes that cell to T cell receptor-mediated stimulation; that is, Nef lowers the biochemical threshold for T cell activation. To elucidate this Nef activity they have developed a murine T cell hybridoma line in which activation, as defined by the T cell specific synthesis of IL2, is dependent upon Nef expression. The Nef-dependent activation pathway was found to require tyrosine kinase activity.

Immunotoxin Induced *In vivo* T Cell Ablation As An Experimental and Therapeutic Tool

Many types of targeted drug delivery systems utilize cell surface receptor binding to achieve targeting specificity. Receptor-mediated endocytosis also provides internalization of the targeted drug. What has not been previously appreciated is the fact that cell surface receptors play an important post-endocytotic role. After endocytosis the receptor ligand interaction determines how the ligand-drug complex will be routed. In many cases this routing uniquely determines the overall efficacy of the drug delivery system. We have shown that a unique anti-T cell immunotoxin, anti-CD3-CRM9, constructed with the diphtheria toxin binding site mutant, CRM9, is highly effective reagent for inducing *in vivo* T cell ablation. The high degree of efficacy and the wide therapeutic margin of this reagent is a consequence of the obligatory intracellular routing pathway required for diphtheria toxin (DT) intoxication. The DT receptor (DTR) typically fulfills this function in diphtheria toxin intoxication. In the case of the anti-CD3 toxin conjugate the routing function is performed by CD3. This epitope routes in parallel with DTR. This fact accounts for the high toxicity of anti CD3 conjugates made with diphtheria toxin binding site mutants such as CRM9. CRM9 alone is incapable of entering the optimal routing pathway and hence has very low systemic toxicity. The anti-CD3 antibody doubly complements the CRM9 defect: It facilitates (1) the entrance of CRM9 into the targeted cell endosomes and (2) directs the entrance of CRM9 into the DT intoxication pathway.

Anti-human CD3 immunotoxins constructed with CRM9 eliminate established tumors of human T cell leukemia in a nude mouse xenograft system. Using an immunotoxin dose set at 1/2 of the minimum lethal dose for CRM9 in sensitive animals, complete regressions were achieved in 80% of the tumor bearing animals. By comparison, 600 cGy of γ radiation from a Cs source were required to induce 80% regressions. Infinite dilution plating assays indicated a 3 log kill

of cells at this dose. In the past this level of response in this type of animal model has been predictive of partial responses and some complete responses in clinical trials. The ability of anti-CD3 based immunotoxins to reduce pan T cell populations *in vivo* provides an immunosuppressive therapy which might be free of the lymphoproliferative side effects which limit current immunosuppressive regimes.

It is generally believed that multiple sclerosis, lupus erythematosus and early onset diabetes are autoimmune diseases and as such would benefit from improved immunosuppressive regimes. Increasingly there are provocative reports which indicate that certain subsets of mental illnesses have an autoimmune component, and these conditions might also be amenable to immunosuppressive therapy. Currently, the ability of anti-CD3-CRM immunotoxins to ablate T cells *in vivo* and produce immunosuppression *in vivo* is being evaluated in non-human primates.

Recently Dr. Marsh's group has found that it is possible to selectively ablate specific Vβ T cell subsets *in vivo* using immunotoxins constructed with specific anti-Vβ antibodies linked to DT. This study has been performed in mice to see if one can eliminate the deleterous response to staphylococcal enterotoxin, a superantigen which interacts and activates a specific Vβ subset. Because specific human immune dysfunction states appear to be associated with specific Vβ subsets, this finding offers a much more selective therapeutic approach. The advantage is that only the offending subset would be eliminated and thymic reprograming of the immune system memory of self versus non-self would not be required (as it would be following total T cell ablation with anti-CD3 immunotoxins).

The Diphtheria Toxin Receptor: Its role in toxin routing and translocation

Previous work in this Section had predicted that the diphtheria toxin receptor was a recycling receptor between the plasma membrane and endosomal compartments. This prediction has now been verified by Dr. Shailubhai. The basis for the prediction was the observation that only certain CRM9 based immunotoxins were toxic and this set included ligand moieties bound to receptors known to recycle. The hypothesis was made that the toxin receptor normally provided a routing pathway which constitutes the obligtory intoxication pathway; CRM9 could only enter this pathway via another recycling receptor. Verification that the DT receptor recycles was achieved by following labeled toxin through internalization and subsequent exocytosis as well as by crosslinking studies of toxin to its receptor on the plasma membrane following exocytosis. In addition, the diphtheria toxin receptor has been found to occupy an asymmetric distribution on polarized epithelial cells. This finding further restricts the possible routes of the toxin receptor recycling pathway and should aid in the morphological identification of this pathway. This knowledge in turn will aid in the rational construction of CRM9 based immunotoxins directed a broader variety of cell surface epitopes and cell types.

ANNUAL REPORT OF THE LABORATORY OF NEUROCHEMISTRY
NATIONAL INSTITUTE OF MENTAL HEALTH
OCTOBER 1, 1991 THROUGH SEPTEMBER 30, 1992

Seymour Kaufman, Ph.D., Chief

For a number of years, the Laboratory of Neurochemistry has focused on two separate, but closely interrelated, research goals: a) the analysis of the mechanisms of the regulation of the biosynthesis of the monoamine neurotransmitters in health and disease, b) the analysis of normal phenylalanine homeostasis and its pathological disturbances - as, illustrated, for example, by events that occur in the genetic disease, phenylketonuria (PKU) - as a model for how the brain interacts metabolically with its peripheral environment. More recently, we have also intensified our efforts to find new roles for tetrahydrobiopterin (BH_4), the essential coenzyme for the aromatic amino acid hydroxylases that was discovered in the Laboratory of Neurochemistry in 1963.

During the last year, we have studied several aspects of the regulation of phenylalanine hydroxylase in vivo. In one of these studies, we uncovered an important mechanism that organisms use to protect the brain against damage caused by postprandial elevations in blood phenylalanine levels. This mechanism involves the phenylalanine-mediated phosphorylation and activation of phenylalanine hydroxylase. This enhanced activity would lead to the more rapid catabolism of phenylalanine, thereby minimizing the chances that the brain could be damaged by the elevated levels of phenylalanine.

We have followed up our observation that cloned PC12 tyrosine hydroxylase, purified to homogeniety from E.coli extracts, is in a highly activated state. One of the manifestations of this activated state is that the cloned enzyme cannot be further activated by what is generally accepted as the main regulatory process for this enzyme, i.e., phosphorylation. This failure occurs despite the fact the enzyme can still be phosphorylated. We have elucidated the underlying basis for the high state of activation of the cloned enzyme by showing that, unlike native PC12 enzyme, the cloned enzyme contains no bound dopamine. Incubation of the pure cloned enzyme with dopamine converts it to a de-activated form that appears to be identical to the native enzyme. Like the latter enzyme, e.g., the dopamine-hydroxylase complex can be activated by phosphorylation. Furthermore, phosphorylation leads to the expected changes in the catalytic properties of the enzyme, including a marked decrease in the Km for BH_4 and a shift in the optimum pH. These studies provide powerful support for the notion that dopamine is a key regulator of the activity of tyrosine hydroxylase.

Studies of tryptophan hydroxylase have lagged behind those of the other two aromatic amino acid hydroxylases, phenylalanine hydroxylase and tyrosine hydroxylase. Part of the reason for this relative lack of information about the properties of tryptophan hydroxylase is that large amounts of the pure enzyme have

never been available. During this last year, we have succeeded in cloning both rabbit and human brain tryptophan hydroxylase and expressing both clones in E.coli. This is the first time that the cloned enzyme has been expressed. This work should enable us to fully characterize this important enzyme.

There have been significant advances in our studies of several new BH_4-dependent systems. We have continued to explore the role of BH_4 in brain nitric oxide synthase (NOS), the enzyme that is responsible for the synthesis of the multifunctional bioregulator, nitric oxide. It was originally reported, by several groups, that constituitive brain NOS differs from the inducible macrophage enzyme in that it does not require BH_4. It was subsequently shown, however, by us and by others that both forms of NOS show similar partial dependence on exogenous BH_4. They are also both absolutely dependent on exogenous NADPH. During the last year, it has been demonstrated that NO synthesis is a two-step process: L-arginine is first converted to hydroxy-L-arginine and in a second step, this intermediate is converted to L-citrulline and NO. Using substrate amounts of brain NOS, we have shown that NADPH is not necessary for the first step, the conversion of arginine to hydroxyarginine. Since this is a hydroxylation reaction, it must consume reducing equivalents. Our demonstration that the partial reaction can take place in the absence of NADPH, raises the possibility that enzyme-bound BH_4 is the electron donor for this step.

During the last few years, it has become clear that activation of the BH_4 de novo biosynthetic pathway is closely associated with activation of the immune system. The basis of the connection between the two processes, however, has remained obscure. One of the possibilities that has been considered is that BH_4 may play some role in the activity of indoleamine-dioxygenase (IDO). Two findings have supported this possibility. A) In immune cells, interferon γ induces both IDO and BH_4 synthesis; B) products of IDO activity play a role in the immune response against certain viral infections. Using several model tissue culture systems, however, we have demonstrated that tryptophan metabolism via the IDO-initiated pathway does not require the presence of any pterin cofactors that are derived from the normal BH_4 biosynthetic pathway.

In our studies of variants of phenylketonuria (PKU), we have carried out biochemical studies that, for the first time, provide an explanation for why patients who suffer from a lack of phenylalanine hydroxylase-stimulating protein (PHS), would exhibit hyperphenylalaninemia (HPA). It is believed that PHS-deficient patients excrete 7-biopterin, an isomer of biopterin, but the possible causal connection between the excretion of 7-biopterin and the observed HPA has not been elucidated. We have now shown that 7-BH_4, the presumed precursor of urinary 7-biopterin, is a potent inhibitor of hepatic phenylalanine hydroxylase. This property, therefore, explains the HPA that is seen in patients who excrete 7-biopterin.

Annual Report

Laboratory of Neurophysiology
Steven Wise, Ph.D., Chief
October 1, 1991 to September 30, 1992

Reorganization of the Poolesville facility

On March 14, 1991, the Scientific Director, in reorganizing the Laboratory of Neurophysiology (LNP), rededicated the Poolesville facility to its original purpose: the study of the neural basis of natural behavior, especially those neglected in traditional neuroscience laboratories. We decided that the LNP would attempt to recruit a recognized leader in the field of neuroethology, a new member of the program whose research would be supported by the laboratory's traditional strengths in neuroanatomy and neurophysiology. During much of 1991 and the first half of 1992, we expended vigorous efforts to recruit a leader for this initiative. To compensate for the intramural program's lack of expertise in the field of neuroethology and in behavioral biology, generally, the Deputy Director convened a Neuroethology Advisory Group consisting of several eminent figures in the field of neuroethology (Mazikazu Konishi, Walter Heiligenberg, Ronald Hoy, and Fernando Nottebohm,) and in its ancestral field, ethology (Peter Marler, John Fentress and Michael Mennaker). This group extended their advice on recruitment and development of an intramural program in neuroethology, and commented on the candidates. A competitive recruitment followed, and a search committee consisting of Mort Mishkin, Chief of the Laboratory of Neuropsychology, Judy Rapoport, Chief of the Child Psychiatry Branch, Ronald Schoenfeld, Deputy Director of the NIMH Intramural Program, and Steven Wise, Chief of the Laboratory of Neurophysiology, selected six candidates for interview. At the time of this writing, May, negotiations continue with the leading candidate. If unsuccessful, we intend to continue the recruitment until we achieve our objective of bringing comparative neurobiology back to the Poolesville laboratory.

Current scientific staff in relation to neuroethology

How compatible is the current scientific staff with the goal of gaining credibility in the field of neuroethology? First, John Olsen, who has a strong background in neuroethological research, was recruited to the Poolesville laboratory in December, 1990. In March, he transferred into the LNP as the first *bona fide* neuroethologist to join the Poolesville laboratory in many years. Second, Josef Rauschecker and Steven Wise, while not dyed-in-the-wool neuroethologists, are neurophysiologists who have strong sympathy for and interest in ethologically oriented approaches. Third, the work of Thomas Insel on affiliative behavior in rodents, and its neurochemical and molecular basis, lies along the mainstream of problems that interest neuroethologists. Finally, the research interests of Brent Stanfield and Chisato Asanuma, while unrelated to the proposed effort in neuroethology, do not oppose it in any way. Indeed, any successful neuroethology program must be supported by state-of-the-art neuroanatomical and neurophysiological technique. Thus, as currently constituted, the Poolesville laboratory offers fertile ground for neuroethology.

Laboratory structure

The newly reorganized Laboratory of Neurophysiology consists of four Units: (1) the Unit on Behavioral Neurophysiology, in essence, the Laboratory of

Neurophysiology as it existed before the reorganization, (2) the Unit on Developmental Neuroanatomy, (3) the Unit on Developmental Biopsychology, and (4) the Unit on Neuroethology. The unit leaders for the first three units are Steven Wise, Brent Stanfield, and Thomas Insel, respectively. The leadership of the fourth unit remains open at this writing. This organizational scheme and its implementation reflects the view that most important scientific work is done by small groups of independent researchers, rather than by a "team" or "center." We emphasize small, "modular" scientific units in lieu of larger groupings that characterize some intramural laboratories. The idea module consists of a senior researcher, one or two junior researchers, and a technical assistant. Administrative support is provided centrally for procurement and personnel actions. The Unit on Behavioral Neurophysiology now has four independent researchers, up from just two in the recent past: Steven Wise, Chisato Asanuma, Roger Erickson, and Donald Crammond. The Unit on Neuroethology has two: John Olsen and Josef Rauschecker, along with our distinguished facility founder, Paul MacLean. Brent Stanfield works with one junior colleague and Thomas Insel collaborates with two junior colleagues. In what follows, work will be attributed to the "submitting author" as defined in the *Guidelines for the Conduct of Research at the National Institutes of Health.*

Unit on Behavioral Neurophysiology. Neurophysiological research projects conducted and completed this year support the hypothesis that the premotor cortex reflects the preparation and selection of voluntary actions, whereas the prefrontal cortex appears to be more generally involved in spatial information processing leading to either perception or movements. Our previous work showed that the premotor cortex plays an important role in behaviors in which a movement must be retrieved from memory on the basis of highly flexible, arbitrary cues. Much of its activity, therefore, depends upon conditional motor learning.

Steven Wise and his colleagues completed a series of studies which established conclusively that apparent visual responses are not strictly sensory, but rather reflect the motor significance of visuomotor instructions. Prefrontal cortex neurons, by contrast, more commonly reflect purely perceptual, attentional or sensory aspects of cortical information processing. Conclusive behavioral neurophysiology, such as that conducted in the LNP, requires the most meticulous attention to detail of experimental design. The two new behavioral methods that had to be invented to address these issues in an awake, behaving animal, required control of spatial attention, memory, arousal, reward contingencies, motivation, and the location of stimuli in retinocentric, craniocentric and allocentric spaces, as well as control of all nonspatial stimulus properties and the response executed by the animal. Wise and his colleagues also tested the important hypothesis, first published in 1983, that cells in the premotor cortex respond to visual stimuli in a given part of space, regardless of where the monkey is looking. This idea is important because it would suggest some simple mechanisms by which the hand could be guided to an object. As attractive as this scheme might be, it is dubious for several reasons. First, the cortical areas that probably provide the visual inputs to the premotor cortex have visual responses that are dramatically influenced by gaze. Second, the hypothesis was based on a poorly controlled study. In our well-controlled study, virtually all cells in both the premotor cortex and in

parts of the prefrontal cortex have apparent visual responses that are affected by gaze angle, refuting the hypothesis.

Chisato Asanuma has continued her work on the fine organization of the neuronal structures involved in regulation of information flow to the cerebral cortex, with particular emphasis on the thalamic reticular nucleus. Her body of work has established many principles of theoretical interest, especially the fact that GABAergic, cholinergic, and adrenergic inputs affect the GABAergic cells of the reticular nucleus. For example, she has shown that the inputs to distal and proximal reticular nucleus dendrites differ and that these dendrites do not make synaptic contacts with each other. These findings provide important clues to the mechanisms by which generalized aspects of brain state, such as sleep, arousal and attention, regulate the activity of cortico-thalamocortical loops and thereby the output of the cerebral cortex.

Unit on Neuroethology. John Olsen has begun an analysis of acoustic information processing in the auditory thalamus of squirrel monkeys. The chuck is a stereotyped call that consists of three components, respectively called the flag, mast, and cackle. Individually, the components resemble other vocalizations. As a neural mechanism for identifying the chuck call, Olsen predicted that there would be neurons sensitive to combinations of the flag, mast, and cackle in the medial geniculate. This idea follows from his thesis work, in which Olsen showed that neurons in the medial geniculate nucleus of the mustache bat responded selectively to combination of information-bearing sound elements. In preliminary results, Olsen found that neurons in the medial geniculate nucleus respond better to combinations of information bearing sounds than to any of the elements alone. The results suggest that combination-sensitivity may be part of a neural mechanism for discriminating acoustically related vocalizations.

Josef Rauschecker has been studying the effects of visual deprivation on auditory cortex and behavior of domestic cats. He and his colleagues have found that animals deprived of normal vision early in life appear to use auditory cues better than sighted animals. In addition, these cats appear to scan their acoustic environment with ear movements and their whiskers grow longer (as do those of visually deprived mice). Along with behavioral changes, the spatial selectivity of neurons in one of the several auditory cortical fields increases, i.e., they respond to sound stimuli over a smaller, more confined part of acoustic space than do cells in normal animals. Neurons in this cortical area, which normally respond to both visual and somatosensory stimuli, also come to respond more to auditory stimuli in visually deprived animals. Rauschecker believes that the changes in sensory response properties may underlie the behavioral plasticity, and he is planning to embark on a series of investigations in unanesthetized cats that test that idea. Rauschecker has also found that cells in the same part of auditory cortex respond selectively to rates of change in stimulus frequency, i.e. tone "sweeps". Such changes in a sound stimulus are somewhat analogous to movement of visual stimuli. At first presentation, this comparison seems paradoxical: how is change in tone of a stimulus (though its source is fixed in space) similar to the movement of a visual stimulus? The answer emerges from consideration of the sensory representations in the cortex. In the visual cortex, the representation of visual stimuli is mapped something like the retina, i.e., in terms of spatial location. Nearby parts of visual space are

mapped to nearby parts of cortex in most visual areas. Similarly, the map in auditory cortex also somewhat resembles its receptor organ, which is organized according to stimulus frequency. Nearby frequencies tend to be represented at nearby parts of the auditory cortex. Thus, in their relation to the topography of their respective cortical fields, movements of visual stimuli can be seen as analogous to changes in tone. Rauschecker believes that feature selection for tone sweeps may underlie prey detection or communication in cats.

Unit on Developmental Neuroanatomy. Brent Stanfield has been studying the principles of neural development, especially of the corticospinal system in rodents. His previous work demonstrated many programmed regressions of neuronal pathways during development. For example, Stanfield and Dennis O'Leary showed that many more cortical areas send axons into the spinal cord in neonatal rats than in adults and many corticospinal axons are lost during development. These phenomena lie at the basis of connectional specificity, the mechanisms by which the precise projections from cortex to spinal cord become established. Stanfield has recently tested the hypothesis that CNS myelin is necessary for the development of normal connectional specificity. Partly because myelin inhibits the growth of developing axons, it has been proposed that myelin may direct late growing fiber pathways. The corticospinal system in rodents serves as a good model system for testing this idea: it develops largely after birth in many rodents and grows into the spinal cord after the ascending sensory fiber pathways have become myelinated. Stanfield has studied *jimpy* mutant mice, which lack CNS myelin because the oligodendrocytes fail to mature. Despite the lack of CNS myelin, the corticospinal system develops normally. Thus, myelin appears to play little role in connectional specificity, at least in this corticofugal projection system. In addition, inputs to the cortex lack myelin and, therefore, the activity of cortical neurons should be highly abnormal. If normal activity plays a role in constricting the number of cortical areas that send projections to subcortical targets, then abnormal inputs might disrupt the patterned regression of these pathways during development. Stanfield's work also shows that CNS myelin plays little, if any, role in this process.

Unit on Developmental Biopsychology. Thomas Insel has shown that the distribution of receptors for affiliation-related peptides, e.g., the nanopeptide oxytocin, correlates with the pattern of social organization in voles. The number of oxytocin receptors in parts of the limbic system of monogamous prairie voles exceeds that of asocial montane voles. At about the time of mating, however, the oxytocin receptor distribution of the solitary voles transiently comes to resemble that of the gregarious voles, especially in the bed nucleus of the stria terminalis and the lateral nucleus of the amygdala. Thus, oxytocin receptor distribution reflects both the overall social variation between species and analogous variation during the life history of a species. Insel has further shown that when oxytocin is given to the monogamous vole, it will form a pair bond (as determined by partner preference tests) even in the absence of mating, which is normally required for pair bonding. This peptide leads to an decrease in aggressiveness in the monogamous voles, but has the opposite effect in the solitary voles. Thus, this particular peptide is sufficient and may be necessary for pair bonding in prairie voles, and also may act to decease affiliative behavior in the solitary species. Insel has also shown that the regulation of oxytocin receptors varies not only between voles,

but among other rodents, as well. Estrogen has dramatically different effects on the expression of oxytocin receptors in the ventromedial hypothalamus in different rodent species: in rats estrogen induces the receptor, in mice it suppresses expression of the receptor, and in voles it has no effect. Insel is pursuing this work in order to understand the underlying molecular mechanisms of this regulation. He has found that, in addition to this steroidal regulation of the oxytocin receptor, the expression of transmitter message itself is regulated by estrogen during lactation. In rats, from birth until postnatal day 4 estrogen inhibits the transcription of oxytocin mRNA in the paraventricular and supraoptic nuclei of the hypothalamus. Thereafter, gene expression is released from such inhibitory control and oxytocin is synthesized in increasing quantities. Eventually, Insel hopes to understand, at the molecular level, the mechanism by which steroidal and other transcriptional cofactors regulate the synthesis of affiliation-related peptides and their receptors.

Significance of the LNP Research Program to the Institute

The Unit on Behavioral Neurophysiology addresses the functional organization of the frontal cortex and the subcortical structures that regulate input to and the activity of the cortex. Our special interest remains on the role of the frontal cortex in motor preparation and motor learning. Functional localization within and among the higher-order cortical fields remains poorly understood, especially in the frontal cortex. Frontal areas are thought to be especially important in the selection and control of the flexible behaviors that characterize advanced primates in their relation to a rapidly changing, but partly predictable, environment. There is increasing recognition that diseases such as schizophrenia, attention deficit disorder (ADD), obsessive-compulsive disorder (OCD), panic and mood disorders, and a variety of dementias, including Alzheimer's, result at least in part from frontal lobe dysfunction (K. F. Berman, B. P. Illowsky and D. R. Weinberger, *Arch. Gen. Psychiatry,* 45 (1988) 616-622; J. A. Mattes, *Comprehensive Psychiatry*, 21 (1980) 358-369; G. J. Chelune, W. Ferguson, R. Kroon and T. O. Dickey, *Child Psychiatry Hum. Devel.*, 16 (1986) 221-233; J. M. Gorman, M. R. Liebowitz, A. J. Fyer and J. Stein, *Am. J. Psychiatry*, 146 (1989) 148-161; H. A. Sackeim, I. Prohovnik, J. R. Moeller, R.P. Brown, S. Apter, J. Prudic, D. P. Devanand, and S. Mukherjee, *Arch. Gen. Psychiatry*, 47 (1990) 60-70; R. M. Cohen, W. E. Semple, M. Gross, T. E. Nordahl, A. C. King, D. Pickar, and R. M. Post, *Neuropsychopharmacol.* 2 (1989) 241-254; S. P. Wise and J. L. Rapoport (1988) In: *Obsessive-Compulsive Disorder in Children and Adolescents,* J. L. Rapoport, ed., Am. Psychiatric Press. pp. 327-344). Some of the views cited above will undoubtedly be proven to be incorrect in attributing these disorders to frontal lobe dysfunction. Nevertheless, the range of these reports shows how important an improved understanding of the frontal lobes of primates will be in understanding a broad range of mental health disorders.

The Units on Developmental Neuroanatomy and Developmental Biopsychology study the rules that guide normal development and the pharmacological mechanisms underlying affiliative behavior, including maternal attachment, separation distress, aggression, and pair bonding. It is likely that the most vexing of all mental health disorders result from abnormal brain development, possibly affecting the limbic system, among other structures. The rules of brain development need to be much better understood if these mental health problems are to be meaningfully approached. The brain mechanisms of affiliative behavior are relevant to a host of mental health problems, from child abuse to autism. Of course, there is a large gap between

the social systems of rodents and humans; the lessons from those species need to be applied very carefully to human health concerns. Nevertheless, the conclusion that a specific neuropeptide transmitter, oxytocin, may be particularly important in affiliative behavior in rodents offers clues for studying brain mechanisms underlying the complex interactions between members of our own species.

The Unit on Neuroethology will be directed by a leader yet to be recruited. For the time being, the workers in this unit are attempting to elaborate the mechanisms of acoustic communication in the thalamus of squirrel monkeys. There is some additional work being done on cross-modal plasticity and auditory localization in cats, but the future of the unit will be in species and neural systems neglected in traditional neuroscience research. The study of brain mechanisms subserving intraspecific communication remains a very attractive area for future development.

Annual Report of the Research Services Branch

National Institute of Mental Health
National Institute of Neurological Disorders and Stroke
Bruce M. Smith, Ph.D., Acting Chief

October 1, 1991 - September 30, 1992

The Research Services Branch provides broad technical support for the Intramural Research Programs of NIMH and NINDS through research and development in advanced biomedical instrumentation techniques and systems and through the evaluation, specification and management of laboratory and office computer systems. The Branch is currently comprised of the Office of the Chief and the Section on Instrumentation and Computers. For this fiscal year, all activities of the Office of the Chief involved the Section projects described below.

SECTION ON INSTRUMENTATION AND COMPUTERS

The Section on Instrumentation and Computers provides technical support for the intramural staff of NIMH and NINDS by: (1) assessing the instrumentation and computer needs of the investigator; (2) designing, developing and constructing special-purpose electronic and mechanical instrumentation systems; (3) designing and specifying laboratory computer systems for data acquisition and processing; (4) designing and developing custom software for scientific and administrative applications; (5) managing a central computer facility in the Weicker Building consisting of a multiuser MicroVAX 3600, an HP 730 UNIX server, an image processing system, and a network of Macintosh personal computers and LaserWriter printers; and (6) developing and managing networks in the Weicker Building and in the Clinical Center. An additional important service provided by Section personnel is consultation on a wide range of topics in the areas of instrumentation, computer science, mathematics and statistics.

When the services of the Section are requested, the investigator first meets with the Section Chief and other appropriate personnel to discuss the requirements. On the basis of this meeting, a decision is made as to whether the Section will take on the project. If a commercial product will satisfy the requirements, the investigator is advised to purchase it. If a custom design is required, we will accept the project unless we lack the appropriate expertise, or our current work backlog is excessive. In these cases, the project may be contracted to a private firm, or the investigator may be directed to the Biomedical Engineering and Instrumentation Program.

When the Section Chief or the Assistant to the Chief agrees to accept a project, a standard Section work request form is initiated. The Section member leading the project then confers with the investigator to formulate a set of specifications and a cost estimate for the project. This information is recorded on the work form which the investigator and his/her Lab Chief sign to authorize the

project. The Section does not charge for services; however, upon completion of the project, the investigator's laboratory or branch is billed for the cost of the components used. Reimbursement of funds takes place at the beginning of the next fiscal year.

INSTRUMENTATION

The Section has a staff of five engineers, five computer specialists and four technicians to design and produce special-purpose instrumentation. It is often appropriate for an engineer, a technician, and a computer specialist to work together to combine electronic and/or mechanical hardware, a personal computer or microprocessor, and custom software, to produce cost-effective solutions to instrumentation problems. The following are brief descriptions of the Section's major projects, taken from a total of more than 300 projects undertaken this year.

Ambulatory Patient Activity Monitoring System. The Section has continued to develop the Patient Activity Monitor (PAM) and the hardware and software which form the system. Intramural investigators and their outside collaborators are using the system in their studies and treatment of depression, hyperactivity, schizophrenia, alcoholism, sleep and eating disorders, and animal models of Parkinson's disease. *Monitor:* Two versions of the PAM are now being supported. The older version has a 1K-byte memory and normally accumulates activity values in 15-minute recording intervals. A new device with a 32K-byte memory and selectable recording intervals of 0.5, 1.0 and 2.0 minutes is now ready to be phased into use. Twenty-five of the new monitors have been produced and are now undergoing final checkout and calibration. *Computer Support*: The Section supports a PAM readout system based on the Macintosh personal computer coupled to a microprocessor-controlled serial interface. A comprehensive Macintosh PAM program handles data readout and disk filing, graphic data editing, construction of continuous data files and plots, and formation of tabular data sets for transfer into spreadsheet and statistical applications. The PAM readout program and the serial interface have been redesigned to accommodate the new 32K PAM and its much higher readout speed, while also maintaining compatibility with the older PAM. *IMS CRADA:* In May, 1989 the NIMH and Individual Monitoring Systems, Inc. (IMS) entered into a Cooperative Research and Development Agreement in order to work together to further the development of the PAM technology for the benefit of the NIMH and the general public. By last year, IMS had produced more than 50 units of the 1K PAM, along with the companion interface units, for commercial sales to research and medical markets. IMS and the Section utilized their joint expertise this year to finalize the design and fabrication methods for the 32K PAM and the new readout interface, and arranged to share the first production runs of the new monitor. Efforts are also continuing to extend the PAM technology to monitor other parameters, such as eye blinks from ambulatory subjects.

Controllers for Perfusion Studies. The Section has continued to make significant improvements to two popular perfusion systems that are used to study the effects of extracellular drug concentrations on cell properties. One system uses a linear-actuator stepper motor to rapidly switch the position of a linear array of nine micropipettes. The solution in each barrel of the array is driven at the same flow rate by a multichannel pump and is gated on and off by a nine-channel valve

controller. Previously, a microprocessor system was developed to provide the precise timing between the stepper motor movements and the gating of the valve controller. The investigator used a personal computer to select perfusion sequences and to download them to the microprocessor. This year, the computer program was greatly expanded to incorporate all the functions of the microprocessor circuitry. A low-cost input/output timer board was used in the personal computer to provide the drive pulses to the motor control chip and to the valve controller. The second type of perfusion system requires no pipette movement. A special micropipette holds nine individual solution tubes whose ends all converge very close to the tiny common exit port at the end of the micropipette. The flow of each solution is again controlled by small valves. A second generation controller was developed last year to provide automatic perfusion sequences and low-power valve switching. The versatility of the controller was considerably increased this year by allowing any combination of the nine nonvolatile programmable sequences to be grouped together and run sequentially. Additionally, the controller was expanded to produce outputs and to accept inputs so that its operation could be synchronized to and/or monitored by other equipment, such as data acquisition or image processing systems. Two of these enhanced perfusion controllers were built, one for the nine-tube micropipette described above, and one for a 12-valve system with independent solution tubes.

Perfusion Pump Controller. In order to carryout expanded experimental protocols with the stepper-motor perfusion system described above, it is necessary to wait 30 seconds or more between perfusion sequences. To conserve valuable drug solutions and to avoid cell damage, the speed of the perfusion pump must be reduced automatically during the inactive time. A programmable timer/speed controller was developed to implement the following functions: accept a trigger pulse from the perfusion controller and wait for a preset time for the perfusion sequence to end; decelerate the pump to a slower speed and wait a preset time; and accelerate the pump back to normal speed just before the next sequence begins. The operator sets the speed and timing parameters with pushbuttons and verifies the settings on a LCD display.

Thalamus Interface. Several intramural labs utilize ISA or EISA computers with custom *Cortex* software for data acquisition, control, and analysis of single unit recordings from primates. A multi-function device, called *Thalamus*, has been developed to serve as an interface between the experimental equipment and the computer's analog and digital input/output boards. *Thalamus* provides convenient interconnection and circuitry for pulse capture (for spike inputs), for pulse generation (for reward delivery), for anti-aliasing filtering prior to A/D conversions, and for touch-pad inputs. A prototype version is currently being tested in the research environment. Since up to ten of these interfaces may be required, a set of five printed circuit boards has been designed to facilitate fabrication and to increase reliability.

Adjustable-Height Elevated Plus Maze. This system was developed last year for studies of anti-anxiety drug effects in mice. The plus maze consists of four equally-spaced elevated runways which intersect directly above an adjustable-height central support base. One set of opposing runway arms are open, with only a small lip on the outside edges. The other set of arms are enclosed on the sides and rear with black plastic. The two open and two enclosed arms are separated by the central intersection area. The transitions from the central area to the four arms are monitored

with double horizontal sets of infrared emitters and detectors. A microprocessor system tracks and counts the animal's entries into the two types of runways and totals the duration time in each type and in the central intersection. Four of these automated mazes were completed this year. A companion printer system was also developed to sequentially print the test results from all four mazes.

Elevated Roller Treadmill. A automated roller treadmill was developed for normal and convulsive locomotion studies in rats. A cylindrical roller with a four-inch diameter is positioned two feet above the base of the device and is divided by adjustable partitions along its length into individual animal sections. The roller is belt-driven by a DC motor whose acceleration, steady-state speed and running time are selected by the user and stored in the microprocessor-based circuitry.

High-Gain Stereotrode Amplifier. To aid ongoing efforts to obtain simultaneous, but distinct single-unit recordings from two neurons in close proximity, a high-gain amplifier system has been designed for use with a new dual-channel metal microelectrode (stereotrode). The system amplifies each signal by a factor of 10,000 with a bandwidth of 300 to 10K Hz. The difference between the two signals is also amplified and filtered with the same specifications. The battery-powered system was designed with emphasis on low-power, low-noise amplification and a high degree of shielding, signal guarding, and RF-suppression on the inputs. Two similar additional models were also developed: a dual version fabricated in the same small package for use with two stereotrodes, and a single stereotrode system with a low-frequency response extended to 10 Hz.

Composite Video Mixer. This project involved development of an interface between a scanning confocal microscope and a video image processing system. The separate, non-standard video signals from the microscope were conditioned and used to generate a composite video signal compatible with the A/D conversion circuitry of the image processing system. A new integrated circuit which combines a video amplifier with a 4-channel high-speed analog multiplexer was used to implement the composite video signal.with proper bandwidth and line-driving capability.

Photon-Counting D/A Interface. Single photon events collected by a photomultiplier tube generate pulses of approximately 100 nanoseconds in duration with an amplitude of 1 mV or greater, and occur at a frequency of 1 MHz or less. An instrument was developed to amplify these events, count them, and generate a proportional 8-bit D/A signal for conversion by a PDP-11 computer. The value of the photon-counting interval is selectable by the user and is synchronized with the computer acquisition system for proper correlation with other acquired signals.

Production of Previously-Designed Instrumentation. Considerable effort was involved in the duplication of instruments and devices that had been previously developed in the Section but were requested this year to satisfy additional needs. The systems that were produced included: 4-channel amplifier/filter systems (2 units); 5-channel variable-frequency pulse generators with gated pulse train capability (3 units); dual-channel sharp-cutoff bandpass filter systems (2 units); a 2-channel variable-frequency pulse generator with high-power biphasic outputs; a 16-channel rat rotometer acquisition system with Macintosh serial interface; a voice-activated switch with computer parallel interface; and a custom two-button reaction time device with computer serial interface. In addition,

an automated light-dark activity apparatus developed more that ten years ago for anti-anxiety drug studies was redesigned this year with current technology. Two of these activity systems were produced for use in the animal facility in the Clinical Center.

NIH Scientific Directors' Voting System. Last year following a special request by the NIH Associate Director for Intramural Affairs, the Section designed a new voting system to be used by the NIH Scientific Directors at their bi-weekly meetings. Fabrication and checkout of the 32 individual voting units, the seven interconnecting junction boxes, the master control unit, and the storage cart were completed early this year. The system has been in routine use since then helping the Scientific Directors efficiently deal with a wide variety of personnel actions. The system provides a high degree of confidentiality and has proven to be easy to use and reliable.

MACHINE SHOP FACILITY

The Section maintains a well-equipped machine shop which is specialized for working with metals and synthetic materials. This facility is critical to the development and fabrication of the electronic and electromechanical instrumentation projects described above. Two technicians also utilize this facility to independently specify, design, and fabricate a wide range of mechanical devices as part of the Section's efforts to provide a spectrum of services in support of basic and clinical research. These staff members are available to advise investigators on mechanical principles and on the properties and uses of materials. Many investigators and other intramural staff frequently come to the shop for immediate help with a small mechanical problem whose timely solution is crucial to their ongoing research. Trained technicians from other labs use our facility to augment the limited capabilities in their own areas.

The following list illustrates the range of mechanical design and fabrication projects typically provided by our machine shop staff: a wide variety of chambers for biological preparations, including tissue cultures, electrophoresis gels, and static and dynamic temperature-controlled perfusion systems; modifications to micromanipulators and to microscopes and other optical devices; modifications to animal chairs, restraining devices and enclosures; pipette holders and storage racks, including radiation shields and collectors; a variety of Faraday cages and enclosures; and numerous adapters for commercial instrumentation.

Our machine shop milling capabilities were significantly enhanced this year by two interrelated developments. First, one of our vertical milling machines was retrofitted with a Computerized Numerical Control (CNC) for automated milling operations in 2.5 axes. Secondly, we purchased a powerful set of Macintosh-based Computer Aided Design/Computer Aided Manufacturing (CAD/CAM) programs which are used to rapidly generate milling programs that are compatible with the CNC machine. The combination of the CNC mill and the CAD/CAM software has been used very effectively by our staff to carryout faster and more precise setups for milling operations, to implement more sophisticated milling operations, and to rapidly duplicate prototype parts.

COMPUTER SUPPORT

In addition to the development of special instrumentation systems, the Section provides support for laboratory and office computer systems and maintains central computer facilities in the Weicker Building for high-capacity data storage, complex off-line data analysis, image processing, scientific word processing, and high-quality printing and plotting. These support services are detailed under the following categories.

LABORATORY COMPUTERS

Small minicomputers and personal computers are widely used in the intramural laboratories for real-time data acquisition and control, mathematical and statistical data analysis, graphics, and word processing. The Section provides consultation on the specification and selection of these systems and helps with the procurement, installation and maintenance of the equipment. Training in operating systems, programming languages, networking and maintenance issues is available for scientists or laboratory support personnel. Within manpower limitations, the Section develops custom software for specific applications. Section computer specialists are always available for consultation and will aid the investigator in writing the difficult time and data dependent sections of real-time programs. Section specialists also evaluate commercial software or programs from other research facilities to determine their utility for intramural laboratory systems.

We have selected the Apple Macintosh family of computer systems as our standard for support of scientific applications. The Section has substantial experience using Macintosh computers to provide solutions for low-speed laboratory data acquisition projects. Two years ago, the Section developed comprehensive specifications for a Macintosh II-based system for the acquisition of real-time data and control of laboratory devices at high speeds. An outside contract was awarded and a versatile program called the Neurophysiological Data Acquisition Program (NDAP) was developed. NDAP was designed to be useful in all disciplines acquiring data, either analog or discrete, in a continuous or event-triggered mode. It contains modules for event-centered graphic displays, signal averaging, baseline reference monitoring, voltage clamping, pre-programming experimental paradigms, maintenance of the experimental logbook, interactive experimental control, apparatus control, and high-speed, continuous data acquisition. Thus far, NDAP has been used primarily in several NICHD laboratories, and is available at no cost for use by all NIH employees.

VAX FACILITY

The Section maintains a DEC MicroVAX 3600 for use by all intramural staff. The 3600 system includes 32 megabytes (MB) of RAM, a 622 MB RA82 system disk, four 664 MB removable SCSI user disks, an Emulex 8 mm 2.3 gigabyte (GB) helical scan tape drive, a TK70 296 MB cartridge tape drive, and a TSV05 1600 BPI tape drive to maintain media compatibility with older systems. VAX/VMS 5.4 is currently installed as the operating system. Pascal and FORTRAN compilers are available for program development. Approximately 50 RS-232-C hard-wired cable

connections are connected to two Emulex P4000 terminal servers, which access the VAX via DEC's Local Area Transport (LAT) protocol over Ethernet. Users can also gain access at 1200 or 2400 baud on five dial-up lines, or at 9600 baud anywhere on DCRT's NIHnet via the Telnet protocol. VAX user accounts have now increased to more than 200.

VAX system software includes AlisaTalk, a package that provides central network file and printing services via AppleTalk protocols to personal computers on the NIH campus-wide network. The Transmission Control Protocol/Internet Protocol (TCP/IP) networking software provides mail, file transfer and terminal sessions to and from a diverse population of NIH campus computer systems as well as the large number of machines on the Internet world-wide network.

The most popular package on the VAX is the sequence analysis software from the Genetics Computing Group (formerly the University of Wisconsin Genetics Computing Group.) This package includes over 100 programs, extensive documentation, and complete on-line help. The Section provides the complete GenBank, NBRF Nucleic, PIR Protein, EMBL and SwissProt databases, and updates all of them quarterly.

UNIX SERVER

To complement the MicroVAX 3600, the Section has purchased a Hewlett-Packard Series 9000, Model 730 workstation to function as a network server. With approximately 20 times the computing power of the MicroVAX, the HP 730 can handle computational-intensive tasks that now take several hours or more. The HP 730 is equipped with 32 MB of RAM, 1 GB of disk storage, and a 1.3 GB DAT tape drive for backup. An additional 16 MB of RAM and 1.5 GB of disk storage will be added in the near future. This new system is connected to the Ethernet portion of the Section's network and through it to the NIHnet and to the international Internet.

As an alternative to the proprietary VMS operating system used on the MicroVAX, the HP 730 provides the UNIX operating system environment that has become popular at the NIH and in the scientific community in general. UNIX provides better support for the TCP/IP file sharing and electronic mail protocols that have become standard on the NIH campus and throughout the scientific community. The Section has taken advantage of the anonymous File Transfer Protocol (FTP) feature on UNIX systems to make software developed by the Section, such as *NIH Image*, freely available to scientists all over the world. The HP 730 has also been set up to function as a Post Office Protocol mail server to provide electronic mail access to the Internet and Bitnet for personal computer users on the NIHnet.

COMPUTER NETWORKS

The Section has continued to expand its network linking Macintosh, Digital, and IBM-compatible computers via the AppleTalk, DECnet, and TCP/IP protocols. The original LocalTalk and PhoneNet network has evolved into a large internetwork including LocalTalk, PhoneNet, a

thickwire Ethernet multiport transceiver, nine thinwire Ethernet segments and a large unshielded twisted pair (UTP) Ethernet star-wired network. The LocalTalk and PhoneNet networks provide low speed (220 Kbits/sec) connections suitable for printing services, Telnet terminal emulation, electronic mail and the transfer of small files between machines. Ethernet's much higher speed makes the transfer of larger files or large numbers of files practical, and is fast enough to allow applications to be shared on a server machine.

There are now 14 LocalTalk/PhoneNet laboratory networks, including two in the Clinical Center, that are linked to the Ethernet portion of the network through nine gateways. The Ethernet portion includes the MicroVAX 3600, the HP 730 server, a VAXStation 3200, a Silicon Graphics UNIX workstation, a Sun SparcStation II, three PDP-11s and numerous Macintoshes and IBM-compatible PCs. The Section provides a Macintosh II AppleShare file server and an AlisaShare file server on the MicroVAX 3600. Several labs run servers on their portion of the network, including AppleShare, LANTastic, and Novell NetWare servers, and many users are taking advantage of the 10-user AppleShare server built into the Macintosh System 7.0 software.

The Ethernet UTP portion of the network was implemented in the Weicker Building this year. The initial installation by AT&T included 64 workstation nodes connected to eight 12-port repeaters distributed in the W-17 wiring closets on each of the five floors. For redundancy, each closet has two UTP connections to the main hub in our central computing facilities on the second floor. Additional nodes and repeaters have been added as needed.

Early this year, the Section participated in a pilot project as the first NIH network to route AppleTalk protocols onto DCRT's NIHnet backbone. Following this successful collaboration, DCRT decided to implement AppleTalk routing throughout the NIH network. Another important development occurred in May of this year when our T1 (1.5 Mbits/sec) link to the campus network was upgraded to Ethernet speeds (10 Mbits/sec).

In addition to these networking facilities and activities centered in the Weicker Building, the Section is involved in a major new effort to implement a PC-based network in the Clinical Center to support the administrative, budget and personnel functions of the NIMH intramural program. An additional computer specialist with the appropriate expertise was recruited to lead this effort. UTP Ethernet wiring and four multiport repeaters have been installed to provide connections for 34 IBM-compatible PCs within the Office of the Scientific Director (OD), the Budget Office, the Administrative Area A, and the Personnel Office. The repeaters have been connected together and to the NIHnet by DCRT's fiber-optic repeaters. Following discussions with DCRT, Microsoft LAN Manager was chosen as the network operating system and has been installed on a Gateway 2000 486 33 MHz server machine. LAN Manager has been installed on most of the PCs and their network connections have been established. The computer specialist has developed and installed on the LAN a database management system for document and correspondence tracking within the OD. Commercial single-machine applications now being used for budget and personnel functions are being evaluated for use on the network. Microsoft Mail will be installed in the near future and will be supported by DCRT on the NIHnet backbone.

Future plans for this LAN include establishing connections to Administrative Area B in the Weicker Building and Administrative Area C at St. Elizabeth's Hospital via the NIHnet, and then making at least one connection to each NIMH intramural lab and branch office.

NIH IMAGE AND THE IMAGE PROCESSING FACILITY

The *NIH Image* processing program for the Macintosh, which has been under development by the Section for almost five years, continues to be popular with scientists in the intramural programs and throughout the world. Important new features have been added in the last year: a routine for removing smooth continuous backgrounds from one and two-dimensional electrophoretic gels and other images; a routine to generate animation sequences by projecting a rotating 3D data set onto a plane; a command for creating composite images from the slices in a 3D stack; and the ability to save selection outlines to disk and restore them later. In addition, macro routines were written for reslicing 3D MRI data sets and for doing cell counting.

Included in the Section's central computer facilities in the Weicker Building is an image processing system consisting of a Macintosh IIfx with 20 MB of RAM and a 19-inch color monitor, a video camera and lightbox, and a digital film recorder for the production of presentation quality 35 mm slides. The *NIH Image* program is used to acquire, enhance, analyze and print images. The facility is useful for numerous applications, including analysis of CT, MRI or PET images, receptor binding studies, analysis of electrophoretic gels, and quantitative evaluation of cerebral blood flow, glucose metabolism, or protein synthesis. Because our facility is based on the relatively inexpensive Macintosh personal computer, and is simple to install and maintain, investigators with extensive image analysis requirements can easily duplicate it for use in their own laboratories.

PERSONAL COMPUTER FACILITY

Also included in the Section's central computing facility are one Macintosh Plus computer, three Macintosh II computers, a Shiva NetModem, three LaserWriter printers, an Apple flatbed scanner with optical character recognition software, a La Cie SilverScan color scanner, and a Montage slide maker. The Macintoshes are connected to the VAX and can be used to emulate VT-100 and Tektronix 4014 or 4105 terminals. A variety of software is available for intramural scientists to use for statistical analysis, for communicating with DCRT's mainframes and MEDLINE, and for word processing, including creation of posters, slides, and publication-quality charts and graphs. Virus detection programs on all the machines have been updated to maintain protection from new computer viruses as they appear. The machines are periodically checked for software copyright compliance, and programs left on the hard disks by our users are periodically purged to maintain compliance. Important additions to this facility that were purchased this year are a Tektronix Phaser III Pxi color laser printer, Macintosh IIfx processor upgrades for the three Macintosh II machines, and a broader selection of Macintosh commercial software to more fully cover the diverse requirements of our users.

DATABASE MANAGEMENT SYSTEMS SUPPORT

One Section specialist works with members of the NIMH administrative offices and the Clinical Director's office, and with other scientific personnel to analyze and define user requirements, and to design, write, and install dBASE III software for database management system applications. Some of these on-going software development projects are described below.

Physician and Institution Referrals. NIMH Clinical Center social workers are frequently asked to provide a list of physicians and/or medical institutions with certain skills and specialties in a region or city in the U.S.A., and in other countries as well. A menu-driven database system has been developed to allow the social workers to efficiently collect and organize this type of data and to quickly respond to requests by rapid searches of the database for relevant information.

Patient Referrals. An extensive system has been developed to handle information regarding individuals who are referred to the NIMH clinical program with a variety of mental disorders. Patients are tracked via this system until such time as they are admitted into, or excluded from, a research protocol. Some patients may be referred to other institutes or to outside physicians. The system is used by the Clinical Director's staff for patient tracking and for the generation of reports and statistical compilations. Further development is planned and the feasibility of implementing this system on the DIRP administrative LAN is being studied.

Clinical Center Patient Costs. This two-phase project was done in collaboration with the NIMH DIRP Budget Analyst. In the first phase, a database system was developed to provide DIRP management with a means of analyzing the quarterly NIH Clinical Center patient cost data. In-patient day and nursing costs for each clinical branch and ward are computed and reported. In the second phase, system specifications were developed and delivered to a contractor to develop a system for the NIH IBM mainframe. The system will track the clinical costs in each branch and will use several different algorithms to project expenditures.

Slides Management System. A menu-driven database system is under development to assist LCB investigators in managing information concerning their tissue slides which are prepared at three different physical sites. The system will enable users to catalog all slides from the different sites, to sort on a variety of categories, including tissue type, animal, and principal investigator, to calculate slide costs, and to produce summary reports.

COLLABORATIVE SUPPORT

Section specialists provide collaborative support for selected research projects within the intramural programs. They provide expertise in computer applications, software development, and statistical analysis and experimental design. These efforts and the resulting software developments are described below.

Extensions to *NIH Image*. Support for research projects in both NIMH and NINDS has led to the inclusion of routines into the *NIH Image* program to count cells and to analyze the spatial

distribution of cells in cultures. In the NIMH studies, the number of cells surviving after pharmacological treatment of a colony is the relevant measure of the effect of that treatment. In NINDS, a direct evaluation of developmental changes in the interactions among cells is required. In both cases, cells are either recognized by appropriate filtering and morphological attribute evaluation or they are manually marked for further analysis. Macro routines count all the cells or count the cells found in randomly-placed rectangles, then produce a list of counts and/or a list of x-y coordinates. In the spatial distribution experiments, further analysis is currently being done using commercial statistical programs. If these methods prove to be of general use, the statistical steps will be added to *NIH Image* as well. In support of a second NIMH study, *NIH Image* is being further extended to control the motorized stage of a microscope to automatically scan a brain section slide. Following a scan, the program will identify and label cells, store their locations and plot an image of the section showing the labelled cells.

Morphological Classification of Cells. In collaboration with two NINDS labs, LNP and LNC, a method of analyzing cell shape has been developed using a Fourier transform of the outline of cells produced by an edge detecting technique. This method is now being applied to studies of neural and glial cell images.

Nonlinear Dynamics in Electrophysiology. The Section is collaborating with Children's Hospital on the application of phase space analysis of the nonlinear dynamics of cells (commonly referred to as chaos theory) as evidenced by transmembrane voltage measurements. Programs originally developed at Bryn Mawr College for the IBM PC were first ported to the Section's MicroVAX. Due to the very long calculation times, these programs are now being ported to the HP 730 workstation. Thus far, studies have involved the analysis of voltage recordings in chick cells exposed to agents which modify lipid metabolism. Two papers are in preparation: one on the methodology and one on the results of the work described above.

Analysis of Rhythmic Phenomena. In collaboration with LCB, NIMH, the Section has begun to develop new methods to describe and fit data to the circadian rhythms of chick melanocytes. These methods involve the fitting of exponentially decaying sinusoidal functions to the biochemical data recorded at intervals over days after various treatments. A major goal is to be able to isolate the effects of the treatments on the phase, frequency and amplitude of rhythms which continue after the cessation of light-dark cycles.

Analysis of Waves of Free Calcium in Glia. The Section has begun a collaboration with LCMN, NICHD, to investigate the nature of apparent free calcium waves in cultured astrocytes as detected by calcium-sensitive fluorescent dyes. The waves can be induced pharmacologically and are quite reproducible within a given cell. Attempts will be made to fit the data to passive diffusion models as well as to regenerative calcium release (*i.e.*, calcium-dependent calcium release). The Section's role will be in the areas of data analysis, model development, and experimental design.

ANNUAL REPORT
VETERINARY MEDICINE AND RESOURCES BRANCH
Division of Intramural Research Programs
National Institute of Mental Health
Gene A. Bingham, D.V.M., Chief
October 1, 1991 - September 30, 1992

The Veterinary Medicine and Resources Branch (VMRB) provides a comprehensive program of animal care and use for the IRP of the NIMH. The program encompasses the use of 263 nonhuman primates, 2 sheep, 48 dogs, 26 cats, 19 rabbits, 148 guinea pigs, 638 hamsters, 578 voles, 80 gerbils, 40,473 rats, 12,942 mice, 4,356 chickens, and 160 frogs, annually. The VMRB operates animal facilities in buildings 9 and 10, at the Neuroscience Center at St. Elizabeths (NCSE), and, as the lead institute, manages the animal care and use program at the NIH Animal Center (NIHAC) Shared Facility. The VMRB is responsible for assuring that the animal care and use programs within the IRP of the NIMH are in compliance with all applicable regulations, guidelines and policies. Additionally, the VMRB serves as a resource to researchers in matters of animal care and use.

PROGRAM

The branch provides 24 hour veterinary medical coverage daily. The staff provides technical training and medical guidance in the care and use of laboratory animals to ensure the effective and humane use of animals in research. The VMRB staff provides for the ordering, receipt and care of laboratory animals in the NIMH facilities in buildings 9 and 10, the NIHAC Shared Facility and at NCSE. One room in building 10 is a shared (NIMH/NIAAA) facility for which NIMH is responsible. The VMRB tracks all animals used by NIMH investigators against the approved protocols. The VMRB staff also ensures that all NIMH animal care and investigative personnel who use animals in their research participate in the animal exposure surveillance program.

The VMRB staff presently consists of 4 veterinarians (VMRB filled the fourth veterinary position this year), 2 facility managers, 2 animal care supervisors, 1 biologist (the executive secretary for the NIMH ACUC and administrative support for VMRB), 1 secretary, 1 veterinary technician, 1 veterinary surgical technician, and 13 animal caretakers [including 3 at the building 36 Central Animal Facility (supervised by NINDS) and 3 contract animal caretakers in buildings 9 and 10]; at the NIHAC facility there is also a contract animal care supervisor and 11 caretakers. The animal care staff and the operation of the NCSE facility formally became part of VMRB this year. Thus, VMRB, in addition to the institute veterinarian, has a facility veterinarian at each of the three geographically different NIMH-operated animal facilities: NIH campus building 9 and 10 facilities, NIHAC Shared Facilities and the NCSE facility.

Branch personnel take an active role in NIH policy forming groups such as the Animal Program Advisory Committee, the NIH Animal Care and Use Committee and its subcommittees, the NIH Nonhuman Primate Plan Steering Committee, and the newly formed Animal Program Director's Committee. VMRB veterinarians serve on the User Committees of several shared/centralized facilities. The NIMH representative on the NIH Biosafety Committee is a VMRB employee.

Administrative support for the NIMH-ACUC is provided by VMRB. This is a critically important position for ensuring that the NIMH operates within the PHS policy and the U.S. Department of Agriculture regulations. NIMH ACUC protocol and program review activities are coordinated within this branch.

The NIMH animal facilities and programs were reviewed by the American Association for Accreditation of Laboratory Animal Care (AAALAC) in November, 1991 along with the other institutes at the NIH. NIH was given provisional accreditation which will be reevaluated sometime in June, 1993. The AAALAC site visitors unexpectedly site visited our animal facilities at the NCSE, and as a result of the walkthrough, an ad hoc subcommittee of the NIMH ACUC, including members of the VMRB, have agreed on an extensive program to have the NCSE facility accredited. This plan is in keeping with the necessity of the NIMH to maintain a research program at this location.

FACILITIES

The planning for building 49 continues. It was determined that the National Eye Institute would be the lead institute for this shared facility. Most of the effort involved planning for the animal caging and for the coordinated occupancy program. The first occupancy for this building is expected in late 1992.

The institute's use of its space in the 10A facility has expanded to its maximum usage. The 4D behavioral suite was competed and usage began in May 1992. However, the NIMH, NIAAA, and NINDS were not alloted additional racks for holding animals in the animal holding rooms that are part of this facility. This has exacerbated our space problem in the 10A facility.

NEUROPSYCHIATRY BRANCH

Richard J. Wyatt, M.D., Chief

INTRODUCTION

The major thrust of the Neuropsychiatry Branch is a continuation of our long-standing efforts to understand the causes of schizophrenia, and to develop treatment and prevention strategies. The theme of our work is best described as the commonly held concepts that schizophrenia is a disorder of limited central nervous system degeneration, of altered development, or of some combination of these. Thus, in addition to studying schizophrenia directly, our Branch has engaged in numerous studies of nervous system development, degeneration and plasticity, and of the factors that may promote or hinder these processes.

COURSE OF SCHIZOPHRENIA

Neuroleptic treatment appears to alter the course of schizophrenia. According to several longitudinal studies, about 50 percent of male schizophrenic patients and 25 percent of female schizophrenic patients exhibit a progressive downward course. What determines this extreme variability is unclear. One factor, rarely mentioned, thought to affect the course of schizophrenia is early somatic treatment. Today, only a few would dispute the evidence that the use of somatic treatments, particularly neuroleptics, decreases the morbidity of acute schizophrenic episodes, and that sustained neuroleptic treatment decreases the risk of relapse. Nevertheless, the question still exists as to whether neuroleptics suppress only the symptoms or whether they affect the course of the disorder as well.

During the last several years we have been reviewing reports that might add information to this subject. We have found a substantial number of studies which, when taken in the aggregate, support the notion that *early* treatment of schizophrenic patients decreases morbidity. Without doubt, certain caveats should be heeded when interpreting these studies, including the complex issue of patient diagnosis, but our findings merit strong consideration nevertheless.

Four groups of studies address the possible long-term benefits of neuroleptic treatment. The first group of studies ("Mirror image") attempted to match similar patients in the pre- and post-neuroleptic eras and compare their outcomes. The second group of studies looked at what happened to patients when intervention with neuroleptics was delayed. The third group examined what happened to stable schizophrenic patients taken off neuroleptics and allowed to have an acute exacerbation of their psychosis. The fourth group (contemporaneous control group studies) made use of patients assigned to neuroleptic or non-neuroleptic treatment groups (usually to examine if

neuroleptics produce a short-term effect over placebo), and followed for a number of years after the experimental period. A summary of the authors' conclusion and the conclusion based upon a reanalysis of the available data is presented in the following table.

Controlled Studies of First or Second Break Schizophrenic Patients Given or Not Given Antipsychotic Medications, and the Effect on Long-term Morbidity.

	Total No. of Studies	No. Relevant Studies	No. with Decrease in Long-Term Morbidity
Authors' (implicit or explicit) View	19	-	11
My View	19	13	13

It can be seen that the majority of studies support the concept that early intervention with schizophrenics affects and improves the outcome. If neuroleptics do influence morbidity, as the studies described above and others indicate, then a mechanism of how they do this can be proposed. Repeated exacerbations of most medical disorders (such as ischemia and inflammation) leave scars. It might then be expected that some biological changes associated with being psychotic also have lasting effects. If, for example, increased dopamine release is associated with psychosis, then our animal model, using repeated injections of cocaine (and the presumed associated increase in dopamine release), could prove useful for exploring brain changes that might be associated with prolonged or repeated psychoses.

A leading study exploring the outcome of early intervention was carried out at Camarillo State Hospital, California in the late 1950s. May and colleagues randomly assigned 228 first-admission schizophrenic patients to one of five treatment groups: milieu therapy, psychotherapy, ECT, neuroleptics, and neuroleptics combined with psychotherapy. In brief, May found that patients receiving neuroleptics or ECT spent much less time in the hospital during their first episode, required fewer days of rehospitalization, and were more likely to be reemployed than any other group. Now, thirty years later, we are attempting a long-term follow-up of those patients in the drug-treated and psychotherapy-treated groups who improved spontaneously within six months of treatment onset. We have, thus far, obtained the hospital records for 12 of the 14 patients treated with psychotherapy alone, and are in the process of finding information on the 65 who received neuroleptics. At first glance, the outcome of the 12

individuals is quite poor; most of these patients have been hospitalized many times since the May study, and have shown little improvement in schizophrenic symptomatology. In the months to come, we hope to follow the neuroleptic-treated patients, and compare the very long-term treatment outcomes of both groups.

ETIOLOGICAL STUDIES

An animal model of the deficit states. Schizophrenia is often associated with productive symptoms, such as hallucinations and delusions, followed by deficit states, such as decreased affect and motivation, and at times dementia. Individuals chronically using cocaine can exhibit both productive and deficit states. For instance, cocaine users often describe seeing visions and other experiences that are similar to the hallucinations experienced by schizophrenics. After the initial intense craving for cocaine following cocaine withdrawal, they often experience depression, apathy, and lack of motivation. Because of the similarities between symptoms of the cocaine withdrawal state and those associated with psychosis, we proposed that cocaine might serve as a model of deficit state schizophrenia. Furthermore, since stimulants, including amphetamine and methamphetamine, given in high doses, are known to decrease dopamine in the brains of animals, we postulated that, in certain brain regions, cocaine might also produce persistent decreases in dopamine metabolism.

In our first study, Drs. Karoum and Suddath administered cocaine to rats in 10 mg/kg doses twice a day for 1 week. Cocaine produced an initial decrease in dopamine in the nucleus accumbens and caudate, but the changes did not persist when cocaine was discontinued. In the frontal cortex and hypothalamus, however, dopamine and its metabolites were decreased for periods ranging from 6 weeks to 3 months following discontinuation of cocaine. Neurotensin, measured in collaboration with Dr. Nemeroff at Emory University, was decreased in the nucleus accumbens 24 hours after cocaine withdrawal. Cholecystokinin was decreased in the frontal cortex, nucleus accumbens and caudate 3 weeks after cocaine withdrawal. Neuropeptide-Y, as well as its mRNA (messenger RNA), measured in collaboration with Dr. Wahlestedt at Cornell University, was also found to be significantly reduced in the frontal cortex, nucleus accumbens and striatum 2 weeks after cocaine withdrawal. Thus, it appears that the effects of cocaine on brain dopamine may be associated with similar changes in those neuropeptides believed to coexist with dopamine neurons, or with neurons that interact with dopamine neurons.

Additional studies were performed to estimate the long-term effects of chronic cocaine exposure on dopamine release and turnover. They confirmed the conclusions derived from steady-state measurements, and revealed new findings that could not be deduced from steady-state studies. Dopamine turnover was reduced during cocaine withdrawal in the frontal cortex, but increased in the

nucleus accumbens and striatum. On the other hand, 3-methoxytyramine rate of formation, or dopamine release, was significantly elevated only in the frontal cortex, an effect that was attributed to long-term reduction in dopamine uptake in this region.

In collaboration with Dr. Deutch at Yale, we found that when the brains of these animals were examined histologically, tyrosine hydroxylase immunoreactivity was quite variable. In a few cocaine-treated animals, there was a profound deficit of tyrosine hydroxylase immunoreactivity in the frontal cortex, striatum and substantia nigra. Poor perfusion cannot completely account for this observed deficit, as Dr. Deutch found normal staining in areas of the brain which also receive dopamine projections.

Using fluoro-deoxyglucose with PET, Dr. Doudet in the Laboratory of Cerebral Metabolism of NIMH found that a rhesus monkey scanned prior to and after receiving 10 mg of cocaine twice daily for 3 weeks had a marked reduction in glucose use throughout the brain. This effect lasted for at least 3 weeks following discontinuation of the drug.

In order to have a measure of the number of dopamine terminals, Dr. Hitri (now at the Washington Veterans Administration Hospital) modified a technique that had been developed for measuring dopamine reuptake sites (transporter) in other brain areas so that it could be used for the frontal cortex of rats and the prefrontal cortex of human postmortem material. The dopamine transporter is thought to be located in the dopamine nerve terminals. In rats, following withdrawal of cocaine, a gradual decrease in frontal cortex reuptake sites ([^{3}H]GBR-12395 binding) developed. Six weeks following discontinuation of cocaine there was a 40 to 50 percent decrease in these sites, and three months following discontinuation of cocaine, the frontal cortex reuptake sites were still below control values. No change in the striatum was found.

Human cocaine abusers also have fewer dopamine uptake sites. Using the assay for dopamine transporters, Dr. Hitri examined dopamine reuptake sites in the brains of autopsied cocaine abusers and controls. The affinity constant for [^{3}H]GBR-12395 was the same for the two groups, but the prefrontal cortex of the cocaine abusers had 34 percent fewer binding sites.

[^{3}H]GBR 12935 Binding Constants in Postmortem Human Frontal Poles Obtained From Controls and Cocaine Abusers

Binding Constants	Controls	Cocaine Abusers	Percent Decrease
K_D (S.E.M.) (nM)	11 (3)	12 (2)	None

Bmax (S.E.M.) (pmoles/mg protein)	3.9 (0.4)	2.5 (0.2)*	36

*p<0.02 (Two-tailed Mann-Whitney test)

Summary table of the [^{3}H]GBR binding constants in the postmortem frontal pole of 13 controls and 13 cocaine abusers. Binding constants were determined from the Scatchard plots using EBDA.

Relationship to schizophrenia. Dr. Hitri has used the same technique she used to examine the effect of cocaine on the brain to determine if schizophrenics also have a decrease in receptor sites. As a group, the prefrontal cortex of schizophrenics had the same number of [^{3}H] GBR 12395 binding sites as the controls. What is notable, however, is that the association between the number of sites and the age of subjects was strikingly different (p=.01). Young schizophrenics appear to have more reuptake sites than controls, while older schizophrenics appear to have less (the correlation with age was -0.51, p=0.004). This suggests that a progressive change with age or duration of illness occurs in schizophrenics, but not in controls. Much work is needed to determine whether the phenomenon is reproducible and clinically significant.

What produces these changes? Studies suggest that the neurotoxicity of other stimulants is the result of their production of the neurotoxin 6-hydroxydopamine (6-OHDA). Pathways for the endogenous formation of 6-OHDA have been proposed. The production of 6-OHDA is thought to be the result of excess dopamine release. Since ample evidence suggests that stress increases dopamine release, particularly in the frontal cortex of rats, and that acutely psychotic individuals are under a great deal of stress, we wished to determine whether 6-OHDA is normally found in the brain; whether its production is responsible for the cocaine-produced changes in the dopamine system; and whether stress might produce similar alterations in brain dopamine systems. Dr. Karoum recently developed a gas-chromatographic/mass-spectrometric assay for 6-OHDA. He found that 6-OHDA is not present in the normal rat brain, but that a substance with a similar chemical structure is present which may have confused previous researchers. Furthermore, 6-OHDA does not appear to be increased by acute cocaine or amphetamines as has been previously reported. We will next see if cocaine's effects are due to the formation of free radicals by administering antioxidants concurrently with chronic cocaine treatment.

Brain Catecholamines. Dr. Karoum and Mr. Oliver, together with Drs. Kleinman and Casanova of the Clinical Brain Disorders Branch, have been measuring catecholamines in brains from autopsied patients and controls. When catecholamines and their metabolites were measured in the cingulate gyrus of brains from schizophrenics and controls, no significant differences were seen for norepinephrine or its metabolites. The dopamine metabolite DOPAC was decreased as was the ratio of molar SUM DA to SUM NE.

Autopsy material: All autopsy brain specimens were provided by The District of Columbia Medical Examiner's Office (DCME). Information about the subjects was obtained from police reports, hospital records and family members. The cause of death was determined by the medical examiner.

Drug analysis. At the time of the autopsy, the DCME routinely conducts drug analyses by gas chromatography of blood, urine, brain, and lung on all subjects including normals. Seven hundred drugs are screened by this method.

Dissection: All brain tissue was dissected manually with a scalpel or other sharp instrument when fresh. An attempt was made to visually separate grey from white matter. The separated grey matter was then frozen and stored at -70°C until it was analyzed.

Biochemistry: Biochemical analysis was performed on a portion of the dissected sample after it had been placed in liquid nitrogen and pulverized. When possible, each tissue was analyzed for dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), norepinephrine (NE), 3-methoxy,4-hydroxyphenylglycol (MHPG), and normetanephrine (NMN). Gas chromatography/mass spectrometry (GC-MS) methods were used to determine concentrations of amines and their metabolites. This method introduces a deuterated internal standard of the substance to be analyzed when the tissue is homogenized, and provides an intra- assay and inter-assay reliability for each substance of greater than ninety-five percent. All assays were performed in a blind fashion, and all assay runs included tissue from schizophrenics, other psychiatric diagnoses, and normals.

Diagnosis: Records from each subject were reviewed by two psychiatrists to provide diagnostic categories according to the Diagnostic and Statistical Manual-III (DSM-III). In doubtful cases, a third psychiatrist was consulted.

Demographics: Cingulate sections were available from 11 chronic, undifferentiated schizophrenics (CUS), 8 chronic paranoid schizophrenics (CPS), 10 suicides, 8 other psychiatric diagnoses and 8 normals. There were 30 males and 15 females. Twelve subjects were known to be taking neuroleptics at the time of death.

Statistics: Comparisons across groups were made by two tailed t-tests. These statistics were calculated for each brain region. The molar sum of dopamine and its metabolites, the molar sum of norepinephrine and its metabolites, and the ratio of these sums were calculated and analyzed with the same statistical methods. When the groups were large enough, relationships to the biochemical measures between taking neuroleptics, and gender were examined. Associations between the time of death and the time the tissue was in the freezer were examined using a Pearson's r for each amine and metabolite. Similar associations were sought for age of subjects at the time of death.

Cingulate gyrus: The following table shows the comparison of all controls with schizophrenics for the catecholamines and their metabolites. The concentration of DOPAC is significantly lower in the cingulate gyrus of the schizophrenics compared with all the controls. A similar decrease was found for SUM DA/SUM NE.

Concentrations of Dopamine, Norepinephrine and their Metabolites in the Cingulate Gyrus of Controls and Schizophrenics.

Substance	Controls		Schizophrenics		
(pmoles/mg protein)	MEAN (SD)	n	MEAN (SD)	n	p<
DA	.941 (.02)	26	0.69 (.48)	19	.4
DOPAC	1.03 (.99)	26	0.44 (.25)	19	.02
HVA	23.0 (6.2)	26	16.7 (8.2)	19	.2
NE	2.83 (2.08)	26	3.46 (1.8)	19	.3
MHPG	.63 (.61)	26	0.73 (.87)	19	.7
NMN	.513 (1.04)	26	0.32 (.38)	19	.4
SUM DA	25.0 (17.3)	26	18.6 (7.8)	19	.1
SUM NE	3.97 (2.64)	26	4.49 (1.9)	19	.5
SUM DA/NE	9.49 (8.71)	26	5.13 (3.4)	19	.05

*Two-tailed t-test.

It appears that DOPAC, and the ratio of DA and its metabolites to NE and its metabolites are decreased in the cingulate of autopsied schizophrenics. There was no difference between schizophrenic subtypes. Postmortem interval and age do not seem to be factors. To the degree that an estimate of the contribution of neuroleptics could be made to the DA differences, they do not seem to be related. On the other hand, there seems to be some effect of neuroleptics on NE.

Plasma Catecholamines. Although there are now numerous technological advances for studying the brain, we are still only beginning to be able to measure functional dopamine in specific brain regions in humans. Until such techniques become more robust, investigators must settle for methods which might provide this information indirectly. Several investigators have found plasma elevations in the dopamine metabolite homovanillic acid (HVA) in drug-free schizophrenic patients, implying that increased plasma HVA might reflect increased dopamine turnover in the brain.

With Dr. Linnoila (National Institute on Alchohol and Alchoholism), Dr. Kirch measured plasma HVA and the norepinephrine metabolite 3-methoxy-4-hydroxyphenyl-glycol (MHPG) in schizophrenic patients withdrawn from neuroleptics. There was a trend for HVA to be increased in these patients, but the only statistically significant increase was in plasma MHPG. Following up on this finding, Dr. Ko (now at Mt. Sinai), Dr. Jimerson, (now at Harvard), and Dr. Bigelow measured MHPG and correlated the concentrations with the clinical state of the patients and their schizophrenic subtype. They found that there was a

trend for MHPG to be elevated in paranoid schizophrenics compared with undifferentiated schizophrenic patients, and for MHPG to be significantly increased in paranoid schizophrenic patients compared with controls. This finding is consistent with other data suggesting norepinephrine elevation in paranoid schizophrenic patients (although it was not found in the cingulate gyrus in our autopsy study).

In addition to an association between MHPG and the subtype of schizophrenia, there also appears to be a relationship between the degree of psychosis and the plasma MHPG concentration. MHPG concentrations were higher when patients had high psychosis ratings than when the same patients had lower psychosis ratings. This finding may be of considerable interest because many years ago, while measuring daily plasma norepinephrine and epinephrine concentrations in patients who had had several acute psychotic episodes, we found that about 3 days prior to any manifestation of psychosis, including sleeplessness and anxiety, resting plasma norepinephrine and epinephrine concentrations rose. These alterations in norepinephrine metabolism are among the findings that suggest the presence of a labile autonomic nervous system in individuals with schizophrenia.

For a number of years, we have worked with Drs. Doudet and Cohen (Laboratory of Cerebral Metabolism, NIMH) to improve the sensitivity of PET scans with ^{18}F-DOPA so that we could examine presynaptic as well as striatal dopamine function. A metabolite of ^{18}F-DOPA, ^{18}F-3OM-DOPA, which is produced in the periphery and accumulates in the brain, is responsible for the non-specific background found in ^{18}F-DOPA PET scans. ^{18}F-DOPA is rapidly cleared from the brain; therefore, it can be prevented from getting into the brain after ^{18}F-DOPA has passed from circulation to the brain (^{18}F-3OM-DOPA is cleared more slowly). It should be possible to measure ^{18}F-DOPA with considerably less background noise than current methods allow. In fact, we found that there was a doubling of sensitivity when unlabeled *l*-phenylalanine was infused after ^{18}F-DOPA has had a chance to accumulate in the brain. While this pilot work was done in monkeys, Dr. Elkashef has begun to examine a group of normals and schizophrenic and Tourette's patients with this technique. Preliminary data look promising.

Excitatory amino acids. Dr. Freed, Chief of our Preclinical Neurosciences Section, has been studying the excitatory amino acids with the specific goal of elucidating the functional role of non-NMDA receptor mediated systems. Non-NMDA receptors may be particularly important in mediating corticostriatal excitatory transmission and, possibly, analogous transmission in the nucleus accumbens. Dr. Freed has conducted a series of experiments with the ultimate goal of elucidating the role of this system in modulating the effects of stimulants and neuroleptics, as well as its role in schizophrenia. Glutamic acid diethyl ester (GDEE), the only pharmacologically useful antagonist of the quisqualate acid type excitatory amino acid receptor, was found to inhibit seizures induced by alcohol withdrawal, homocysteine administration, and intraventricular quisqualic acid.

This compound also was found to attenuate the stimulant effects of amphetamine, PCP and apomorphine. NMDA antagonists did not produce similar effects.

Because GDEE may spontaneously hydrolyze in solution, and because its potency is very low, Dr. Freed has also begun to look for alternative compounds with similar pharmacological properties in collaboration with Dr. Rzeszotarski of George Washington University. Encouraging initial results have been obtained with some of these compounds.

Dr. Freed has noted that, in patients, the administration of neuroleptics may have a gradual onset, and their maximum effect develops over several weeks. In order to understand this effect, he has carried out a series of experiments on neurotransmitter receptors following chronic neuroleptic treatment. Dr. Freed has noted an intimate association between glutamate-mediated corticostriatal and dopamine-mediated nigrostriatal pathways. He has been investigating the interaction between the glutamate and dopamine systems by evaluating changes in AA2 excitatory amino acid receptors following administration of haloperidol and other neuroleptic medications, in rats and in postmortem human brain. Some changes in the high-affinity AA2 receptor in postmortem samples from schizophrenic patients have been found in the caudate nucleus, but not in samples from the human cortex. This change is currently being studied in an additional, larger study.

Second Messenger System Studies. In recent years, interest in the mechanism of action of neuroleptics and other psychotropic drugs has been extended to include their effects on intracellular second messenger systems, including phosphoinositide turnover. Drs. Li and Kirch have now completed a series of three studies making use of a phosphoinositide hydrolysis assay to assess the effects on turnover in rats chronically administered a variety of drugs. In an initial study, chronic haloperidol was found to decrease carbachol-induced stimulation of phosphoinositide turnover. These results are similar to the effect of lithium. In a second study, three drugs with anti-manic efficacy, lithium, haloperidol, and valproate, were found to attenuate phosphoinositide turnover, with differences in brain regional specificity and time course. This finding may be highly relevant to their common clinical efficacy despite the fact that they are from disparate chemical classes. A third study has shown that nicotine, a drug which is commonly used by psychiatric patients, also affects phosphoinositide turnover.

Cerebrospinal Fluid Studies in Schizophrenia. Previous studies attempting to analyze cerebrospinal fluid (CSF) from patients with schizophrenia and controls have suffered in that only one or a few compounds have been quantified at a time. Typically, when several different compounds have been measured, different assays are required and are performed under variable conditions at different times. Thus, when correlations and comparisons between multiple

compounds in patients and controls are made, the technique does not account for problems related to inter-assay variability. We now have the capability to perform a multidimensional analysis of CSF using multichannel high performance liquid chromatography to determine whether significant relationships exist among these compounds, which include neurotransmitters (catecholamines and indoleamines) and their metabolites thought to be implicated in schizophrenia. Drs. Kirch and Issa, in collaboration with Dr. Gerhardt at the University of Colorado, have analyzed CSF samples obtained over a four-year period. Discriminant function analysis of nineteen monoamines and related compounds identified tryptophan, tryptophol and epinephrine as factors discriminating between drug-free patients and normal controls. A subgroup of patients studied on versus off neuroleptic treatment had higher CSF MHPG in the latter state. There were no significant correlations between the off neuroleptic CSF concentrations of any of the monoamines and ratings of severity of psychopathology. On the other hand, off medication 3-hydroxykynurenine and kynurenine predicted the outcome of treatment in a multiple regression model. In a subgroup of patients studied on versus off neuroleptic treatment, there was no correlation between the change in CSF monoamine concentrations and outcome of treatment with a standard dose of antipsychotic. MRI data were obtained on some of the patients.

Drs. Egan and El-Mallakh have looked for evidence of neuronal degeneration in patients with schizophrenia by assaying levels of neuron-specific enolase (NSE) in CSF. NSE is a neuron-specific protein released during neuronal cell death that is elevated in a number of neurological and neurodegenerative disorders. So far, no evidence of increased NSE has been found in acute or chronic schizophrenia. In fact, there is a suggestion that NSE is decreased. Interestingly, neuroleptic treatment may produce a normalizing effect. This work will be replicated over the next two years as additional CSF is collected.

Adding on to the neuron-specific enolase work done last year, Dr. El-Mallakh hopes to measure brain-type creatine kinase (CK-BB) in the cerebrospinal fluid. The presence of this otherwise intracellular enzyme in the CSF would indicate cellular membrane disruption. A sensitive ELISA assay is currently being developed in collaboration with Dr. Larry Tamarkin of Assay Research.

In related work, Drs. El-Mallakh and Wyatt collaborated with Dr. Paul Cervey of Rush Medical School in Chicago to measure a dopamine-responsive neurotrophic factor (DRNF) in the CSF of schizophrenic patients. DRNF is elevated in the CSF of Parkinson's Disease patients and is believed to reflect an attempt of the damaged dopaminergic system to repair itself. No differences were found between schizophrenics and controls, or between medicated and unmedicated schizophrenics. The latter result suggests that the assay, in its current form, is not sensitive enough to detect the elevation of DRNF that would be expected to occur in response to neuroleptics. Attempts to increase assay sensitivity are underway.

Interleukin-2 (IL-2) is a cytokine with growth-promoting properties which has been reported to be elevated in the CSF of schizophrenics. A larger and more careful study was undertaken by Drs. El-Mallakh and Wyatt, with the same ELISA assay used by other investigators. It was found that the concentration of IL-2 present in the CSF of humans is too close to the detection limits of the assay. Consequently, variance in split samples is comparable to reported differences between schizophrenics and controls. The possibility of using a more sensitive bioassay is being investigated.

Growth Factors in Spinal Fluid from Schizophrenic Patients. Previously, we noted that CSF from schizophrenic patients promotes the growth of chick dorsal root and sympathetic ganglia cells. The data shown below, expressed as the percent of surviving neurons when no trophic factor is added, are the results of CSF added to chick sympathetic ganglion cell cultures.

Neurotrophic Activity in CSF as a Function of Diagnostic Group

Diagnosis	n	Neurotrophic Activity (% of control cultures) mean (SEM)
Normal volunteers	20	93 (4)
Neurologic contols	18	115 (12)
Non-schizophrenic psychiatric patients	7	124 (11)*
Schizophrenic patients	29	173 (15)**

* $p<0.01$; ** $p<0.001$ when compared with controls.

We observed a correlation between the growth-promoting effect of the CSF of patients with schizophrenia, and the size of their lateral ventricles, as measured on CT scans. Unfortunately, the previous assay proved to be unreliable because the response was not always present. To develop a more reliable assay, Mr. Oliver has modified a neuroblastoma cell line (Neuro 2-A) assay used by others for a similar purpose, and has found it to be sensitive to small quantities of growth-enhancing substances. Growth-enhancing substances were found in the CSF of both chronic schizophrenic and normal control individuals, but there was no difference in activity between the two groups.

Alpha-2FS. Alpha-2FS haptoglobin is a phase reactant (ct is elevated during the active process of certain diseases) protein which we, with Drs. Ginger Johnson and Carl Merril of the Laboratory of Biomedical Genetics and Dr. Daniel D. VanKammen of the Pittsburgh University, have found to be elevated in the CSF of a group of chronic schizophrenic patients and patients with Alzheimer's disease. Interestingly, the patients with schizophrenia have higher CSF concentrations than those with Alzheimer's disease. During the next year, we

will be working with Dr. Merrill's group to determine the significance of this finding.

The Hydra Head Activator Peptide. The Hydra head activator peptide is important in the development of the head and nervous system of the multi-celled organism *Hydra attenuata*. This 11-amino acid peptide is also found in the human brain. Drs. Quach and Duchemin, now at Ohio State University, have shown that the Hydra head acivator peptide, in concentrations as low as 1 pM (a concentration that causes bud and limb formation in the hydra), increases survival of cultured chick embryonic sympathetic and dorsal root gangion cells. It also induces rapid morphological differentiation of the mouse neuroblastoma cell line Neuro-2A. Within 4 hours of its addition to a culture of Neuro-2A cells, the cells responded with process extension. The observation that this peptide, which is conserved phylogenetically from the most primitive nervous system known (the coelenterate) to the human brain, suggests it plays an important role in the development of the nervous system.

Neuropathological Studies. Dr. Stevens has previously noted gliosis in periventricular and other areas of postmortem brains of many schizophrenic individuals. These findings have potential pathophysiological implications both for etiology and for defining the time of onset of the illness. Some investigators, however, using other techniques, have not been able to show gliosis; thus, the presence of gliosis in brains of schizophrenic individuals is controversial and it is important to resolve this question. With Dr. Casanova (Clinical Brain Disorders Branch), Dr. Stevens has reported a substantial difference between two staining techniques (using an antibody to glial fibrillary acid protein [GFAP] and the Holzer's method) in the detection of chronic fibrillary gliosis. To pursue this question further, on a blind basis, she examined a new series of schizophrenic and control brains in collaboration with Drs. Bogerts and Falkai (University of Dusseldorf, Germany) and Dr. Clive Burton, Ramwell Hospital, Essex, U.K. This study has not shown increased gliosis on gross inspection of whole brain sections stained with Holzer's technique for glia.

Drs. Poltorak, Stevens, Casanova (Clinical Brain Disorders Branch) and Freed have been studying phosphorylated neurofilament (PNF) epitope expression in rodent and human brains. PNF immunoreactivity reportedly was not found in neuronal cell bodies except under some pathological conditions in which abnormal accumulation of neurofilaments induces neurofilament phosphorylation in the perikarya of neurons (e.g. Alzheimer's disease). They have found that PNF immunoreactivity is located in the perikarya of normal neurons and is not exclusively present in the axons, a finding that contradicts earlier reports. Furthermore, they found that PNF immunoreactivity is present in the hippocampal pyramidal cells of autopsied brains from schizophrenic individuals as well as controls. As described by others, they found PNF immunoreactivity in the plaques and tangles of brains from patients with Alzheimer's disease. Finally, they found that the method of preparing tissue for

fixation alters the staining properties of monoclonal antibodies directed against phosphorylated epitopes of neurofilaments. This may explain some of the differences between Dr. Poltorak's studies and previous efforts, and indicates that neurofilaments may be more susceptible to disruption by fixation when in the perikarya.

In current studies, Drs. Stevens, Poltorak and Casanova are examining immunohistochemical markers for tyrosine hydroxylase, synapsin, synapsophysin, and PNF in the nucleus accumbens of schizophrenic and control postmortem specimens. Studies have shown a trend for fewer axons in schizophrenics than control brains, and a change in pattern in one dimension - PNF positive.

In another study, Dr. Poltorak found that PNF immunoreactivity of primary and secondary dendrites of Purkinje neurons was reduced in mice prenatally exposed to ethanol. By 2 months of age, however, the PNF immunoreactivity appeared to be fully developed in the ethanol-exposed mice, suggesting that there is a maturational delay in animals that have been prenatally exposed to ethanol.

Dr. Stevens, in collaboration with Dr. Bruton, neuropathologist, Ramwell Hospital, U.K. and Chris Frith, Northwick Park, London is studying neuropathological changes in postmortem brains of epileptic patients with and without psychoses (n=57). Preliminary findings were presented at the International Congress on Schizophrenia and at the International Congress of Biological Psychiatry. Final results were presented at the International Epilepsy Congress in The Netherlands in April 1992. Material is being prepared for publication.

Drs. Duchemin and Quach (now at Ohio State University), together with Drs. Iadarola and Naranjo (now at the Dental Institute), found that cholecystokinin (CCK) immunoreactivity and CCK messenger RNA (mRNA) are often located in different parts of the brain. Cholecystokinin may have an eightyfold difference in parts of the brain where the CCK mRNA variability is only fivefold. The highest concentrations of brain CCK mRNA are in the cortical, hippocampal, and thalamic, areas where CCK-containing cell bodies appear to be located. Cholecystokinin is found in very high concentrations in areas of the striatum and ventral striatum where no detectable CCK mRNA is found. The implication of these findings is that, although CCK is synthesized in the cell bodies, it is most heavily concentrated in terminals some distance away.

Drs. Kirch, El-Mallakh, and Kleinman (Clinical Brain Disorders Branch), in collaboration with a group of neuropathologists, conducted a quantitative analysis of the basal forebrain cholinergic system in the nucleus basalis of Meynert. They found no difference in the numbers of neurons (or numbers of cortical plaques) in schizophrenic patients with prominent cognitive deficits. In contrast, patients with senile dementia of the Alzheimer's type showed the

expected decreased cell counts and increased plaques. Thus, even with pronounced cognitive impairment, schizophrenic patients do not show significant basal forebrain pathology.

^{18}F-DOPA and Cerebral Blood Flow PET studies. Working with Drs. Doris Doudet and Robert M. Cohen (LCM, NIMH), we have found a large asymmetry in the striatum/cortex ratios and uptake rate constants between MPTP-injected and non-injected sides of hemi-parkinsonian animals. These PET studies were carried out up to 25 months after the last dose of MPTP, and demonstrated that the effects of this neurotoxin are lasting. We have also demonstrated, using a technique that reduces the background noise by about 50 percent, that there is reduced DOPA uptake in the striatum of the side not injected with MPTP, suggesting that MPTP is not completely trapped in the striatum during the first pass. In these studies, CSF homovanillic acid (HVA) and, to a lesser extent, CSF 5-hydroxyindoleacetic acid (5HIAA) were strongly correlated with ^{18}F-DOPA PET data. The lower 5-HIAA suggests that MPTP partially destroyed serotonin neurons in addition to those containing dopamine. Surprisingly, neither spontaneous nor apomorphine-induced turning correlated with ^{18}F-DOPA PET data or CSF HVA content. While there were no abnormalities in cerebral blood flow in these animals, the striatum on the lesioned side did not have low blood flow. On the unlesioned side, the blood flow was high, suggesting a compensatory mechanism was present.

Neuroleptic medications appear to increase brain dopamine in the synapse. For many years, it has been known that neuroleptic drugs increase dopamine turnover within the brain. Over 25 years ago, it was postulated that neuroleptics increase dopamine turnover by blocking postsynaptic dopamine receptors, thereby decreasing the effectiveness of a negative feedback loop initiated by neuroleptics. Blocking of the dopamine receptors has traditionally been considered the principal mechanism of action of neuroleptics, especially since a number of studies indicate that the initial increase in dopamine turnover subsides following prolonged neuroleptic administration. A well-regarded explanation for this down-regulation in dopamine metabolism is that, in time, neuroleptics block depolarization and decrease firing of dopamine neurons. Nevertheless, most biochemical studies which have examined dopamine turnover following chronic administration of neuroleptics indicate that the developing tolerance is not complete (i.e., dopamine turnover does not completely return to baseline). Traditionally, investigators of this phenomenon have examined the dopamine metabolites homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC). Neither of these metabolites, however, directly reflects the action within the synapse. This is important since the increased HVA and DOPAC production may be related to a change taking place inside the neuron, and may not reflect the amount of dopamine available to the postsynaptic receptor.

In order to better understand this phenomenon, Drs. Karoum and Egan, following up on the work of others, developed a new method to measure the small amount of dopamine released within the synapse. First, they developed an extremely sensitive assay for measuring the dopamine metabolite 3-methoxytyramine (3MT). Three-methoxytyramine is believed to be produced after dopamine interacts with a postsynaptic neuron, since the enzyme (catechol-O-methyl transferase) which forms 3MT is bound to the postsynaptic neuron. To measure the small quantity of 3MT in specific brain regions, Dr. Karoum developed a chemical ionization technique for the gas-chromatographic mass-spectrometer. This assay is extremely sensitive and allows quantification of 3MT and other biogenic amines when their concentrations are low, and the amount of tissue to be examined is small. Since 3MT is rapidly produced after an animal dies, to accurately measure the metabolite, the enzyme forming it must be inactivated at the time of death.

An assay using whole brain microwaving that rapidly inactivates enzymes which might alter the formation and degradation of neurotransmitters was developed a number of years ago by the Neuropsychiatry Branch together with the Preclinical Pharmacology Laboratory, at that time directed by Dr. Costa. We have again used this technique to measure 3MT production following neuroleptic administration in rats. After acute administration of the typical neuroleptic haloperidol, there was a rapid rise in 3MT in the rat striatum and frontal cortex. Clozapine, an atypical neuroleptic, produced no change in 3MT production in the striatum, but increased 3MT production in the frontal cortex. This may explain the clinical observation that clozapine has few extrapyramidal side effects. What is of more interest, however, is that following administration of haloperidol to rats for 28 days, a partial tolerance developed in the frontal cortex. Tolerance was not observed in the striatum. Following chronic administration of clozapine, there was greater tolerance in the frontal cortex and, once again, clozapine produced little effect in the striatum. The failure to find a consistent elevation of 3MT in the frontal cortex suggests that increased functional dopamine within the synapse is not responsible for the effect of neuroleptics.

Drs. Karoum and Chrapusta have explored the role of neuronal impulse flow on dopaminergic transmission and its interaction with neuroleptics. The rate of formation of 3-MT was used as an index of dopamine release, and gamma-butyrolactone, as a chemical agent that blocks dopamine neuronal impulse flow. Employing this approach, they were able to characterize the presence of an impulse flow independent release of dopamine in the rat brain. This impulse flow independent release of dopamine was found to prevail more so in the frontal cortex and nucleus accumbens than in the striatum. In the hypothalamus, this type of dopamine release constituted over 75% of the total release of dopamine. Furthermore, while both acute and chronic neuroleptics markedly elevate impulse flow dependent release of dopamine, the impulse flow independent counterpart was not affected.

Stress and the brain response. Recent clinical studies indicate that stress can exacerbate the symptoms of schizophrenia, and that introducing measures which decrease stress can prevent relapses. Dr. Gilad has been examining the effects of stressful stimuli on two inbred strains of rats which differ in their reactivity to stress as well as in their span of life. He has found that an ongoing, age-dependent degerneration of septohippocampal cholinergic neurons is associated with two compensatory changes: The cell bodies of remaining cholinergic neurons increase in size, and presynaptic cholinergic terminal markers increase in activity, which suggests that there may be collateral sprouting of the cholinergic terminals. Dr. Gilad has also noted that an age-dependent loss of cholinergic neurons in the septum precedes loss of hippocampal pyramidal neurons. Finally, the loss of pyramidal neurons in the hippocampus is associated with a compensatory increase in muscarinic binding by the remaining hippocampal neurons. Reactive changes in neurotransmission are not limited to cholinergic neurons. It is conceivable that within a given responsive neuronal network, several components react to create a new state of activity. Indeed, our recent studies indicate that glutaminergic neurons increase their activity in response to stress in a region selective manner. Thus, within the septal-hippocampal-septal circuit, both cholinergic and glutamatergic neurons are activated in response to stress. This activation does not appear to be secondary to the activation of the hypothalamic-pituitary-adrenocortical axis and rising levels of glucocorticoids. Rather, it is probably dependent on the activation of ascending neuronal inputs, such as dopamine neurons that terminate in the septum. In addition to finding changes in neurotransmission functions, Dr. Gilad has found alterations in protein patterns on two-dimensional gel-electrophoresis immediately after stress.

Since alterations in the hippocampus have been described in schizophrenia, and since it has been well-established that stress can precipitate relapses in both affective disorders and schizophrenia, the interaction between stress, aging, and Dr. Gilad's neuropathological findings are of considerable interest. He has found similar changes in the frontal cortex, also an area of interest for schizophrenia.

In a recent series of studies, Dr. Gilad examined the effects of various regimens of lithium chloride treatment on dexamethasone-induced increases in brain polyamine metabolizing enzymes. In contrast to peripheral tissues where acute lithium treatment suppresses the increase in polyamine biosynthesis, in the brain, only chronic treatment was effective in preventing this increase. These findings indicate a novel brain target for lithium's action and, in turn, provide new avenues for exploring polyamine function in manic-depressive illness.

VIRAL AND IMMUNOLOGICAL STUDIES

There is a growing body of evidence that suggests that schizophrenia may represent a viral or immunologic disorder of the brain. For instance,

schizophrenia-like illnesses have been reported to occur during or after a variety of viral infections, such as influenza, epidemic encephalitis, and herpes simplex. Other studies document an 8 percent increase in the birth rate of future schizophrenics during the first quarter of the year, and a 7-10 percent rise in hospital admissions in the late spring and early summer. Several members of our branch have been involved in testing for the presence of viral infection or autoimmunity in patients with schizophrenia.

Schizophrenia, Viruses and Immunology. To determine whether there is an association between schizophrenia and retroviruses, Dr. Feenstra (now at Heidelberg University), Dr. Kirch and Mr. Coggiano measured reverse transcriptase activity in lymphocytes of patients with schizophrenia. In their first study, 17 patients with chronic schizophrenia were compared with 10 normal controls. Peripheral blood lymphocytes were stimulated with phytohemagglutinin (PHA) and cultured in the presence of T-cell growth factor. Supernatants from the cultures were collected at 3 to 4 day intervals. No significant differences were found in reverse transcriptase activity between patients and controls. In the same study, no difference in reverse transcriptase activity was found between patients taking neuroleptics and those not taking neuroleptics. Using similar techniques to culture lymphocytes and stimulate them with 5-azacytidine (a compound widely used to activate viruses), Dr. Feenstra and her colleagues again found no difference in reverse transcriptase activity when schizophrenic patients were compared with normal controls. In another attempt to determine if there is retroviral activity in lymphocytes of patients with schizophrenia, Mr. Coggiano found no reverse transcriptase activity in response to stimulation by gamma irradiation.

Dr. Stevens and Dr. Schwartz (NINCDS) have found that CSF taken from chronic schizophrenic patients at the Neuropsychiatric Research Hospital causes neuroblastoma (SH-EP) cells in tissue culture to grow to a higher density as compared with CSF from neurologic controls. Possible transformation of these cultures has been demonstrated by testing colony formation in soft agar. The CSF from the schizophrenic patients promoted increased culture size and/or number of colonies compared with the CSF of controls. The "agent" which produced the increased density and colony formation has continued its expression in new cultures of neuroblastoma cells following 30 passages of cell-free media. The "agent" is capable of passing through a .45 micro/m filter and remains transmissible after it is diluted 10^{-8} fold. Dr. Stevens and her collaborators, Drs. Schwartz Susumu, and Sherabe, found no evidence of the presence of reverse transcriptase, oligoclonal bands, lectin surface markers, or schizophrenia-related protein on 2D gels. Electron microscopy revealed Bovine Serum virus (a contaminant) in both schizophrenics and controls. The agent responsible for cell transformation can be inactivated by temperature, proteases and nucleases. The DNA extracted from the cell-free media culture and from affected and unaffected neuroblastoma cells has been examined on gels. The agent has been isolated to

one band on a Percoll Gradient and this band has transformed new cells. Cloning and sequencing are in progress.

In a second study, Dr. Stevens and Ms. Phillips (Clinical Brain Disorders Branch) inoculated newly born mice (n=107) intracerebrally in fresh or frozen CSF from patients with acute or chronic schizophrenia (n=14) and controls (n=13). Systematic behavior tests were done on these animals at 2 months of age. Animals were sacrificed if sick or at 1 year after inoculation if no symptoms developed. A histologic examination of brains has been completed "blind" to type of CSF inoculated. Mice inoculated with fresh (but not frozen) CSF 1 year earlier had larger ventricles compared to other groups.

A more general test for the presence of possible viral infection and/or autoimmunity in the central nervous system of patients with schizophrenia is to measure the specific concentration of immunoglobulin G (IgG) in cerebrospinal fluid (CSF), correcting the value for individual differences in blood-brain barrier permeability. Drs. Kirch, Suddath, Alexander, and Papadopoulos (Clinical Chemistry Service of NIH) measured CSF and serum albumin and IgG in schizophrenics and controls. Simultaneous measurement of serum and CSF albumin and IgG allows one to quantify the endogenous IgG production in the brain and is a potential indicator of infection and autoimmunity in the central nervous system. Expanding upon a previous study, Dr. Kirch and colleagues found evidence of increased central nervous system IgG production in 20% of schizophrenic patients. In addition, there was evidence in one patient of oligoclonal (abnormal) banding when electrophoresis of the CSF was performed. It is also of interest that 22% of patients showed signs of increased blood-brain barrier permeability.

In a similar study, in collaboration with Dr. Papadopoulous, Dr. Stevens reported no evidence of oligoclonal bands in 8 out of 9 patients with early or acutely exacerbated schizophrenia. The one patient with oligoclonal bands also had a positive serum test for antibodies. There was no evidence for intrathecal IgG production in patients with schizophrenia.

In another study, Drs. Alexander, Kirch, and Spector (University of California at San Diego) used the polymerase chain reaction (PCR) technique to search for viral DNA sequences from cytomegalovirus (CMV) in postmortem brain tissue from schizophrenic patients. CMV infection has been implicated as a possible etiologic factor in schizophrenia. Genomic DNA was isolated from the brains of 8 schizophrenic subjects, 8 non-schizophrenic suicide victims, and 8 normal controls. CMV-specific amplification was not detected in any of the brains studied.

In a related study, conducted by Drs. Kirch and Alexander in collaboration with Dr. Murray (Rocky Mountain Multiple Sclerosis Center), PCR for DNA from herpes simplex virus type I (HSV-I) was performed on the same samples. No

evidence of HSV-I DNA was found in these samples, although control brain tissue samples from patients with herpes encephalitis were positive.

Drs. Glovinsky, Kirch and Kulaga are also currently using the PCR gene amplification technique to search for the presence of DNA from human T cell lymphotrophic virus (HTLV-1) in postmortem brain tissue from patients with multiple sclerosis and control subjects. Multiple sclerosis is another neuropsychiatric disorder for which, like schizophrenia, a viral etiology has been proposed. Several discrete, high intensity bands consistent with some homology to the target HTLV-1 on fragments appear to have been found both in the brains from patients with multiple sclerosis and control subjects. These fragments represent endogenous, retrovirus-like sequences. In addition, RNA studies suggest that these endogenous sequences are expressed in a number of tissues.

HLA Studies. For the past 10 years, scientific reports from Italy have indicated that chlorpromazine interferes with the action of alloantibodies directed against human leukocyte antigens (HLA). Specifically, the Italian studies provide evidence that chlorpromazine inhibits binding of anti-HLA-A1 and HLA-A1-related antisera to lymphocytes with HLA-A1 antigens. Dr. Alexander, attempting to replicate this work, found that chlorpromazine does not inhibit the cytotoxic action of anti-HLA-A1 sera, but is cytotoxic to peripheral blood lymphocytes in concentrations greater than 1.5×10^{-5}M.

Previous studies have also reported an association between specific HLA antigens and schizophrenic subtypes, as well as correlations between HLA antigens and structural brain abnormalities found on computed tomography (CT). Dr. Alexander studied 55 Caucasian chronic schizophrenic patients and looked for clinical associations at HLA-A and B loci. After correcting for the number of statistical tests performed, he found no differences in antigen frequency betwee schizophrenic patients and controls. In addition, no significant differences in antigen frequencies were found between controls and subgroups of schizophrenics (based on clinical subtypes), response to neuroleptic treatment, size of the lateral ventricle, size of the parieto-occipital sulci, or reversed frontal or occipital asymmetry as found on the CT scans.

Plasma Interferon. Drs. Kirch, Preble, and Torrey measured alpha interferon in paired plasma samples from chronic schizophrenic patients both on and off neuroleptic medication. The patients as a group had a modest increase in plasma interferon titers compared with controls. The presence of medications did not appear to significantly affect interferon titers. This study confirmed a previous study suggesting that schizophrenic patients as a group have modestly elevated interferon titers, and indicates that this elevation is not simply a neuroleptic effect. Since interferon is a potential measure of viral infection and/or autoimmunity, the finding of increased interferon further supports the association between schizophrenia and either viral infection, autoimmunity, or both.

HIV-1 Studies. Dr. Kulaga, in collaboration with colleagues at the National Institute of Allergy and Infectious Disease, found that rabbits can be infected with HIV-1 by injection of human T cells harboring the virus. The identity of the virus was demonstrated by restriction enzyme mapping and by infectivity of isolated virus for human cells. It was also demonstrated that infected animals carried the virus in the brain. Virus could be detected over a considerable range of timepoints post inoculation. Immune responsiveness measured in HIV-1 infected rabbits was drastically diminished in these animals when compared with controls.

This year, Drs. Truckenmiller and Kulaga, in collaboration with colleagues at Nova Pharmaceuticals and Johns Hopkins University School of Medicine, has found that a human cortical neuronal cell line (HCN-1A) can be infected with HIV-1 isolated from brain cells. Virus could be detected over a considerable range of time points post infection. Flow cytometric and antibody blocking experiments have shown that infection does not proceed through a CD4-mediated pathway. A variety of in vitro studies indicate that infection may be mediated through viral binding to Gal/C, a molecule with a somewhat restricted tissue distribution pattern.

In studies to date, approximately 300 rabbits have been infected by the previously described protocol, and analyzed by the preceding test procedures. Ninety percent of animals singly inoculated with HIV-1 infected A3.01 cells have been confirmed as seropositive by ELISA. A major difficulty with using the rabbit as an in vivo system was addressed in the lab this past year. Specifically, the minimum rabbit infectious unit was found to be 10,000 infectious units as determined by an indirect plaque assay of material used for inoculum. Bleedings taken from these rabbits at two week intervals following infection were tested by ELISA for the presence of antibodies to HIV-1 proteins. Serum samples were scored as the percent of the optical density (OD) value of the given positive control. All values were calculated after the subtraction of background OD values; preinfection serum from the rabbits was used as a negative control to ascertain baseline reactivity. Samples giving 30 percent of positive control values were considered positive. Samples which were positive by ELISA were then tested by Western blot analysis using strips of nitrocellulose containing isolated HIV-1 proteins and developed with gold-labeled goat anti-rabbit antibodies. Ninety three percent of rabbits infected with A3.01 cells were positive by ELISA; samples tested by Western blot analysis were also positive for antibody directed against a number of HIV-1 proteins.

Nucleic acid samples from tissues obtained at necropsy were subjected to PCR analysis, and a summary of these experiments is available upon request. Samples were amplified with synthetic oligonucleotide primers with sequences based on the reported sequence of the HIV-1 (LAV) isolate used in these studies. Primers were derived from both gag and env regions of the genome with sequences that

spanned 574bp and 389bp respectively. The validity of the fragment obtained by 30 cycles of amplification was shown, not only by its conformity to the expected size, but also by the presence of restriction enzyme sites in the positions expected from the published sequence. A given tissue is only scored as positive after Southern blot analysis of gels used to separate the products of the reaction. These blotting studies used probes from the HIV-1 genome.

The studies described above use basic techniques in immunology and molecular biology. Flow cytometry is used to measure cellular compartments that react with specific monoclonal antibodies. In order to investigate the genetic control of changes in immune responsiveness, inheritance and/or expression patterns of several genes known to play important roles in a variety of immune processes will be analyzed.

During this past year, several new projects concerned with changes in peripheral blood mononuclear cell (PBMC) populations were instituted. The objective of these investigations is to identify the characteristics of lymphocyte subpopulations in the following systems: (1) Rabbits experimentally infected with HIV-1 and HTLV-1 (Definitions of alterations in rabbit PBMC populations will enhance the value of this species as a model for various human diseases that have an effect on the immune system); (2) Humans multiply exposed to HIV-1 who remain seronegative and PCR-unreactive (The apparent ability of a few individuals to resist infection with HIV-1 points to important differences in their immune system. Determination of the factors conferring this apparent "resistance" are of obvious importance); and (3) Individuals with psychiatric disorders. Autoimmune mechanisms have been postulated to play a role in the pathogenesis of schizophrenia. It is possible that a subgroup of patients are afflicted due to an underlying autoimmune or genetic cause. Alternatively, administration of antipsychotic drugs may alter lymphocyte population in a subgroup of individuals. Preliminary clinical data confirm that the percentage of CD5 positive lymphocytes (a cell type associated with autoimmunity) is increased in schizophrenic patients. We have also noted, however, similar increases in other diagnostic groups, especially patients with substance abuse. A basic science study has yielded highly relevant findings, insofar as work by Dr. Wing has shown that chronic administration of haloperidol and/or nicotine, both of which are drugs commonly used by schizophrenic patients, may alter a number of lymphocyte subsets.

Dr. El-Mallakh has been doing preliminary work to evaluate whether the psychosis and mania that occassionally occur in dementia associated with the acquired immunodeficiency syndrome (AIDS) may serve as a model for schizophrenia. Some of this early work suggests that individuals are predisposed to psychosis early in the course of dementia, and enter a permanent remission as the dementia becomes more severe. This is qualitatively similar to the course of psychosis and dementia in poor prognosis schizophrenia.

Development of Rating Scales. Since its inception, the Neuropsychiatry Branch has been involved in the development of behavioral rating scales for quantifying observations of schizophrenic patients. These include numerous modifications of the Brief Psychiatric Rating Scale (BPRS) and Abnormal Involuntary Movement Scale (AIMS), as well as the development of the Premorbid Adjustment Scale (Cannon-Spoor) and Negative Symptom Rating Scale (NSRS). Our recent attention has focused on the NSRS. We have found that the NSRS measures defect signs and symptoms which appear to be independent of positive symptoms in schizophrenia. Using the NSRS, we examined the development of defect symptoms in our patients and found that these symptoms appear to increase in proportion to the duration of the patient's illness. We also found no relationship between defect symptoms and tardive dyskinesia, but did find a strong inverse relationship between current IQ, as measured by the WAIS-R, and defect symptoms. It is of considerable interest that in the latter study we found no relationship between defect states and premorbid social functioning, as measured on our Premorbid Social Functioning Scale.

Dr. Taylor has created an extensive developmental data base from information gathered with the Neuropsychiatric Developmental Questionnaire (NPDQ), which is completed by the parents of each patient entering the Neuropsychiatric Research Hospital. The 470-item instrument has been computerized and explores the following areas: pregnancy, birth and delivery, medical history, social-behavior patterns, education, environmental and economic influences, and family medical and mental health histories. A Personality Inventory for Children (PIC) is also completed by the parents. This 600-item behavioral observation scale has been found to have strong construct and discriminant validity. By combining the PIC and NPDQ, a comprehensive family, social, behavioral and medical profile can be systematically constructed. This information will be used in an attempt to make associations between premorbid history and current biological measures. To date, developmental histories of 85 inpatients have been collected and entered into our data base.

Schizoaffective Disorder. Because patients with schizoaffective disorder display a wide variety of psychotic and affective symptoms, we believe that a better understanding of this population may provide clues to affective illness, schizophrenia, and the psychotic process in general. Research on this population has generally been hampered by poor clinical classification. Consequently, it was imperative to collect a well-defined, carefully diagnosed cohort of schizoaffective patients. Dr. El-Mallakh began by creating a computerized, 50-item symptom checklist that incorporates all the diagnostic criteria of three diagnostic systems, the DSM-III-R, the RDC, and the Maj & Perris. This checklist has already shown good validity, compared to the SCID, in preliminary testing, and early phases of inter-rater reliability testing also look good.

Schizoaffective patients, so categorized, were asked to enroll in one of 2 studies. One project involved the use of clonidine, administered in a double-blind, placebo-controlled fashion, to control symptoms of hostility, agitation, aggression, and irritability. The other involved screening and identification of a subset of psychotic patients that may have elevated urinary levels of phenylethylamine (PEA), a normally occurring trace amine. These patients will eventually be asked to participate in a double-blind, placebo-controlled clinical trial of carbidopa, a peripheral L-amino acid decarboxylase inhibitor.

Dr. El-Mallakh's investigations regarding the use of clonidine as an adjunct treatment for schizophrenia and schizoaffective illness have been disappointing. Patients treated with clonidine did not differ on behavioral and tardive dyskinesia ratings from those given placebo. The study has been terminated, and a paper describing the total experience with 15 patients is being prepared.

Screening for patients with elevated phenylethylamine (PEA) continues. Results with the initial 40 patients revealed one patient with greatly elevated PEA (25 x normal), and one with moderately elevated PEA (8 x normal). Both refused treatment with carbidopa. Collaborations with Dr. Fenton at Chestnut Lodge and Dr. Post at NIMH promise to greatly increase the patient population. A modification of the carbidopa study design has been submitted to the IRB which will hopefully increase the attractiveness of the study to potential participants.

The Homeless Project. For a number of years, we have stressed the importance of experimentally testing large-scale social interventions influencing psychiatric patients with the scientific scrutiny applied to biological interventions. For example, prior to the large-scale dismantling of the state hospitals, no well-controlled studies performed in this country had demonstrated either the feasibility or the consequences of deinstitutionalization. Even today, only a few good experimental studies have examined how to deinstitutionalize subgroups of patients, but too few studies have been undertaken to make generalizations across communities and patient populations.

The recognition of the extent of homelessness amongst the seriously mentally ill has prompted a number of institutions and governments to sponsor surveys and demonstration projects. With few exceptions, none of these demonstrations has been experimentally tested with a well-controlled design. With Dr. Caton, Mr. Grunberg, and Dr. Felix at the College of Physicians and Surgeons at Columbia University, we are involved in a pilot study to demonstrate the feasibility of performing experimental studies of the mentally ill homeless. We have examined a group of homeless men participating in a Psychiatric Shelter Program operated by the Department of Psychiatry at Presbyterian Hospital, New York City. The program offers day treatment services to men housed at the Washington Heights Armory Municipal Shelter on 168th Street. A multidisciplinary team provides psychosocial counseling, recreational therapy, medication maintenance, and housing placement to men with psychiatric

illnesses. Approximately 75 men are enrolled in the program at a given time, with an average length of stay of about 5 months. The goal of treatment plans fashioned with the patient's participation is to help patients develop their social skill level (i.e., their ability to relate to mental health professionals and to each other), and to help them cope with various daily pressures in ways that do not perpetuate their condition.

Two studies have been performed. The data for the first was collected during the summer of 1988, and for the second, early in the winter of 1992. Results of the first study are briefly described: All individuals placed in the program from its inception in the summer of 1986 were studied until January of 1988. Their life situations 6 months before entering the program were compared with their life situations 6 months after termination. Of the 200 men who attended the program, 50 were involved long enough to undergo discharge planning, 39 were placed in community housing, 8 were transferred for psychiatric reasons to inpatient care, and 3 were transferred to other shelters for the homeless.

An interview schedule was developed to elicit social background data and information on living arrangements, financial support, psychiatric inpatient/outpatient treatment, employment, and criminal justice contacts in the 6 month periods prior to the program, and following discharge to community housing. DSM-III diagnoses were made based on case records, and a clinical assessment was performed by the program's psychiatrist. Ninety-four percent of the men were single, 87 percent were unemployed, and 78 percent were Black or Hispanic. The median age was 32 years, 63 percent were high school graduates, 25 percent had some college experience, 19 percent had served in the armed forces, and 44 percent had jail or prison histories. Eighty percent had had at least one prior psychiatric hospitalization, schizophrenia and schizoaffective disorder being the most common psychiatric diagnoses. Simultaneous substance abuse was noted in 63 percent of the men studied, and poly-drug abuse was frequent.

The outcomes of the before-and-after evaluation were quite positive. While 4 persons spent time living on the streets in the 6 months immediately before entering the program, none of the subjects did so in the period after. Four persons had criminal arrests before entering the program; only two were arrested after the program. Twenty persons lived in shelters for the homeless in the pre-program phase, but only three did so afterward. In fact, the total number of days spent in shelters was reduced sevenfold. In the phase before the program, 10 subjects participated in psychiatric aftercare treatment, while after it, 20 subjects participated. The total number of aftercare visits increased threefold from 286 to 843. At the end of the study, all of the patients had some income. Psychiatric hospitalization slightly increased from a total of 724 days to 802 days.

One of the most notable findings of the study concerned the rehospitalization rate after the program: 78 percent of the patients who returned to their family

living settings had to be rehospitalized in the 6 months following involvement with this shelter, in contrast to 30 percent of those living in their own apartments, and 8 percent of those in community residency. We are now one year into our first randomized control study to determine the effectiveness of the methods we have developed for placing formerly homeless individuals and helping them keep their housing. At the time of writing this report, about 70 individuals have entered the study.

In January, 1992, the last of the 18-month, post-discharge interviews was completed. Unfortunately, the early positive effects of the program seem to have been eroded by time. Forty percent of the program participants had become homeless again, and criminal justice contacts increased to a number exceeding that in the before-teatment phase (although when adjusted for the length of pre- and post-study periods (6 versus 18 months), the number was less. While employment increased from its pre-program level of 4 to 10, the number of men without income approached that found in the pre-program phase. Of the 12 men who experienced psychiatric hospitalizations in the 6 months before the program, 10 were hospitalized in the 18-month follow-up period. Nine of the men hospitalized at any time in the after phase had not been hospitalized in the before phase. Those participants with drug abuse diagnoses who were discharged to their own apartments or to a family setting, as opposed to a community residence, were most likely to re-experience homelessness at both the 6-month and 18-month follow-up points.

Pharmacokinetics of haloperidol. Dr. Kirch has continued to examine the relationship of blood haloperidol and metabolite concentrations to the clinical response of schizophrenic patients. Past studies have indicated that patients have a response threshold of approximately 5 ng/ml of serum haloperidol. Above 5 ng/ml, patients appear to have an increasing sigmoid-shaped response curve with little, if any, further clinical benefit above 15 ng/ml. This work, using a fixed dosage of haloperidol (0.4 mg/kg/day), indicates that there is no "therapeutic window" for haloperidol as some reports in the literature suggest, and Dr. Kirch's work confirms that, for most patients, there is no clinical advantage to attaining higher blood concentrations. A replication of this study was done with Dr. Potkin, now at the University of California at Irvine, as part of a multi-site World Health Organization study of patients who were given a fixed dosage of haloperidol. Analyses of these data again indicate that patients gain no additional benefits when blood concentrations climb above a certain level. In order to determine the appropriate dose of haloperidol for a given patient, Dr. Kirch has given patients a single dosage of haloperidol and measured the peak serum concentration, which occurs at about 3 to 5 hours. He has found a strong correlation between the acute peak concentration and the ultimate steady state haloperidol concentration following daily haloperidol doses, suggesting that in the future it might be possible to predict the dose of haloperidol that a patient may need by measuring the serum haloperidol concentration following a single dosage.

Interactions of haloperidol and other drugs. Several drugs have been noted to alter serum haloperidol concentrations. For example, using a fixed dose of neuroleptic, Dr. Kirch has found that smoking tends to lower serum haloperidol concentrations. It is of interest to note that when the study sample was limited to patients who were smokers, there was no significant difference in haloperidol concentrations between patients with and without tardive dyskinesia. Drs. Kirch and Straw noted that retinoic acid also lowers the serum concentration of haloperidol in patients. The retinoic acid finding is of particular interest because in the one patient in whom retinoic acid produced the greatest decrease in haloperidol concentration, Dr. Straw noted clinical improvement of psychosis. This did not generally occur in a larger patient group. To follow up the patient studies, Dr. Straw examined the effect of 13-cis-retinoic acid on the concentration of haloperidol and its metabolite, reduced-haloperidol, in the rat. Paradoxically, serum haloperidol and reduced-haloperidol concentrations were increased following the administration of retinoic acid. Dr. Straw, now a guest researcher, is attempting to determine the reasons for the difference in haloperidol-retinoid interactions between species.

Drs. Straw, Bigelow and Kirch demonstrated that ascorbic acid administration does not affect serum haloperidol concentrations and does not enhance the clinical response in patients with schizophrenia, as might have been expected from several animal studies and an earlier study of patients.

With Dr. Ko, Dr. Kirch measured the concentrations of haloperidol and its reduced metabolite in the plasma and red blood cells of our patients. They found that red blood cell haloperidol concentrations do not predict a patient's clinical response any better than the plasma concentrations. However, they did find a preferential uptake of the reduced metabolite into red blood cells, which indicates that tissue stores of the reduced metabolite may be very high. We have previously shown that the reduced metabolite of haloperidol is relatively biologically inactive, but it is unclear what proportion of reduced haloperidol is converted back to haloperidol in man.

Calcium Channel Inhibitors. For several years we have been examining the effects of calcium channel inhibitors on the behavior of schizophrenic patients. In the past, we found that the calcium channel inhibitor verapamil produced no change in our chronic schizophrenic patients. In animal studies, however, we found that nifedipine-like drugs (dihydropyridines) inhibited PCP (phenyclidine)-induced and amphetamine-induced behaviors in mice better than verapamil. Drs. Suddath, Straw, Freed and others have studied the effects of the dihydropyridine calcium channel inhibitor nifedipine in 10 chronic schizophrenic patients, 4 of whom had tardive dyskinesia, in an 8-week, double-blind, cross over trial. Nifedipine had no effects on the symptoms of schizophrenia. In the 4 patients with tardive dyskinesia there was an average improvement on the total Abnormal Involuntary Movement Scale (AIMS) of 57

percent, and a trend toward overall worsening of psychotic symptoms in these patients. While this trend did not reach statistical significance, a statistically significant decrease in abnormal movements in our patients was noteworthy, for it suggests nifedipine's potential for treating tardive dyskinesia.

Dr. El-Mallakh has published a theoretical paper on the mechanism of action of calcium channel blockers in bipolar illness, and has continued his work on the role of ions in mania and bipolar illness. This has led to one paper investigating reasons for differing levels of susceptibility to lithium toxicity in children, adults, and the elderly. Another paper outlining the Na, K-ATPase hypothesis for bipolar illness has recently been completed.

MAO Inhibitors. Attempts to use dopamine agonists to alleviate deficit state symptoms have resulted in a global worsening of symptoms rather than an improvement. Administration of *non-selective* MAO-inhibitors to schizophrenics has also been contraindicated. To study the effects of augmenting central dopamine activity on negative symptoms, Dr. Sambunaris has proposed a sixteen-week, double-blind, placebo-controlled trial of selegiline given to patients on a stable dose of neuroleptics. It is hoped that selegiline, a *selective* MAO-B inhibitor that increases the amount of dopamine, will ameliorate negative symptoms without concurrently exacerbating the positive symptoms and side effects associated with typical non-selective MAO-inhibitors. Testing will begin once the final IRB approval of the protocol is obtained.

TARDIVE DYSKINESIA STUDIES

Neuroleptics and Tardive Dyskinesia. To better understand the epidemiology of TD, we are studying the relationship of neuroleptic dose to the development of abnormal movements. While the length of time an individual is on neuroleptics appears to correlate directly with the risk of developing tardive dyskinesia, it is uncertain whether the dose of neuroleptic is also a factor. To understand this problem, Drs. Ko (Mt Sinai), Zhang, Yan, Zhang, Buchner, Xia, and Jeste studied 866 inpatients at the Shanghai Psychiatric Hospital where the doses of neuroleptics used are considerably lower than we are accustomed to in the United States. This study was effected by a scientific collaboration between the U.S. State Department and the People's Republic of China.

Each patient's dose was kept consistent for at least 1 month before the data was gathered and throughout the course of the study. 8.4 percent (73 patients) of the patients had tardive dyskinesia as compared to a prevalence range of 13 to 25 percent in the United States. The mean daily dose of neuroleptic in chlorpromazine equivalence was 311 mg, which is considerably lower than the usual dose given in the United States. Our findings are consistent with reports from the United States, Japan, and India associating a lower prevalence of tardive dyskinesia with the use of smaller amounts of neuroleptics. Surprisingly, we did not find that clozapine protected the patients from tardive dyskinesia. Clozapine

has been widely used in China for over 10 years, and it is possible that the patients who developed tardive dyskinesia were switched to clozapine or were selectively placed on it in a manner which would bias our study. The relationship of clozapine to the development of tardive dyskinesia is now being examined prospectively.

Dr. Khot published an important epidemiological study determining the actual risk of developing tardive dyskinesia for patients who take neuroleptics over long periods of time. Patients who are likely to take neuroleptics are also likely to develop spontaneous movements which are phenomenologically indistinguishable from drug-induced dyskinesia. It is important for patients and their families to know the risk of developing drug-induced dyskinesia as they consider treatment alternatives. Dr. Khot surveyed studies in which identical rating instruments (such as the AIMS) were applied to patients who had been on neuroleptics and to patients who had not. Since age is an important factor in the development of abnormal involuntary movements, he subdivided patient groups by age. He found that there is relatively little risk of patients under 30 years of age developing spontaneous movements, but this risk increases with age. Similarly, the risk of developing neuroleptic-related movements increases with age. After about 40 years of age, the occurrence of spontaneous dyskinesia is high enough to make it impossible to determine if the abnormal movements of any individual patient are drug related. In fact, for some age groups it appears that the risk of developing spontaneous dyskinesia is actually somewhat higher than for drug related dyskinesia. This not only has importance for patients and their families, but also has potential medico-legal implications.

Laterality and Tardive Dyskinesia. Dr. Egan, together with Drs. Hyde, Kleinman and Weinberger (CBDB) studied 158 patients with tardive dyskinesia to determine what percentage of patients had lateralizing movements. Previous studies have suggested that a neuropathological insult in schizophrenia may be lateralized. Reports of asymmetry in tardive dyskinesia have been used to bolster this hypothesis. Surprisingly, most patients were found to have asymmetric movements. This was not dependent on the psychiatric diagnosis of the patients: There was no preference for one side over the other. In a subgroup of the patients examined repeatedly over 12 weeks, the asymmetry was found to fluctuate. At least four ratings were necessary to accurately predict the presence of sidedness of persistent asymmetry.

Nicotine and Tardive Dyskinesia. Schizophrenic and other psychotic individuals hospitalized for long periods of time are often observed to be heavy smokers. Dr. Kirch has found that smoking was significantly more prevalent among patients with schizophrenia than among patients in other diagnostic categories. Furthermore, patients with schizophrenia who are smokers are more likely to have tardive dyskinesia than nonsmokers. Working with Dr. Benowitz at the University of California at San Francisco, Dr. Kirch and Dr. Suddath found that

patients with tardive dyskinesia have higher concentrations of both nicotine and caffeine in their blood than patients without tardive dyskinesia.

In order to determine the effect of nicotine on dopamine, Dr. Kirch, with Drs. Gerhardt and Freedman at the University of Colorado, demonstrated that nicotine given for 3 weeks appears to decrease dopamine turnover in the striatum, frontal cortex, and hypothalamus of the rat. Caffeine, a drug often concurrently used by psychiatric patients, was shown to have a similar effect on dopamine turnover in rat brain with chronic administration. Dr. Wing has recently accumulated data on neurochemistry and behavior in rats chronically administered haloperidol and nicotine alone and in combination, which indicate that nicotine initially potentiates haloperidol-induced decreases in behavior, but that tolerance develops to this effect over time.

Dr. Kirch, with Dr. Creese at Rutgers University, measured the concentration of D1 and D2 receptors in the rat striatum following daily administration of nicotine and caffeine for 28 days. He found that neither drug caused a significant change in D1 or D2 receptors, suggesting that the relationship of these agents to tardive dyskinesia is not caused by dopamine receptor upregulation.

Drs. Khot and Kirch examined the relationship between serum haloperidol, smoking, and tardive dyskinesia. They found that serum haloperidol concentrations were significantly lower in smokers than in nonsmokers. Subjects with tardive dyskinesia had significantly lower concentrations of reduced haloperidol and a trend toward lower haloperidol concentrations. The trend toward lower neuroleptic concentrations in subjects with tardive dyskinesia did not change when analysis was limited to smokers.

Relationship between neuropsychological deficits and tardive dyskinesia. During the last year, Drs. Egan, Gold, Goldberg and Kirch have provided very strong evidence that relatively young schizophrenic patients with tardive dyskinesia do not have more neuropsychological deficits or CT head scan abnormalities than a similar group of patients without tardive dyskinesia. They matched 32 patients with schizophrenia who had been diagnosed as having tardive dyskinesia during their stay at the NIMH Neuropsychiatric Research Hospital with a group of 32 patients of identical age, gender, and educational background who did not have tardive dyskinesia. Each patient received extensive neuropsychological testing as well as a CT head scan. No significant differences were found in any of the variables relating to the neuropsychological testing, including Full Scale IQ, Verbal IQ, and Performance IQ. None of the subscales of the WAIS were different between the two patient groups. Furthermore, subscales on the Halstead Reitan Battery were not different between the groups. Measurements of the ventricular-brain ratio (VBR) were also not different between the groups. This study demonstrated that, at least between ages 18 and 41, dyskinesia is no more likely to occur in patients with neuropsychiatric deficits or abnormal CT scans than it is in patients without these changes. Perhaps of more importance, it suggests that

within this age range the factors that produce tardive dyskinesia--most specifically neuroleptic medications--do not also produce cognitive or gross structural deficits in the brain.

To further investigate the possibility of neuropsychological impairment, we have given patients with tardive dyskinesia the pursuit rotor test. This test evaluates procedural learning, a function that depends on the integrity of the striatal "motor loop" as described by De Long et al. We will also attempt to evaluate the oculomotor striatal loop using eye tracking in collaboration with other NIMH investigators. These studies will help to tease out the degree of functional impairment of the motor striatum in patients with TD and may help to identify other striatal informational loops that may also be impaired.

Drs. Egan and Gold are also exploring the degree of psychopathology and response to medications in patients with TD compared to those without TD. The former group appear to have prima facia evidence for increased striatal dopaminergic tone, which accounts for the dyskinetic movements. It is unclear from the literature whether or not these patients are more psychotic, or whether they respond differently to treatment. In a retrospective study, Drs. Egan and Gold are looking at measures of psychosis in patients both on and off neuroleptics, as well as the degree of improvement on medication in schizophrenic patients with TD, compared to those without TD.

Vitamin E and tardive dyskinesia. A few years ago, we noted that vitamin E, when given to rats, reduced the manifestations of IDPN-induced (IDPN is a neurotoxin) dyskinesia. Subsequently, we gave vitamin E to a group of patients with tardive dyskinesia and noted a significant (43 percent) reduction in abnormal movements. Dr. Egan has once again confirmed this finding in patients with TD for short periods (5 years or less) who improved an average of 18.5 percent. While the clinical effects were not dramatic, this does support the free-radical hypothesis of TD. This finding was replicated by Dr. Elkashef prior to his arrival at NIMH. In fact, in 4 out of 5 studies, vitamin E has been found to be helpful in the treatment of tardive dyskinesia. We plan next to use a more potent antioxidant, coenzyme Q, in a clinical trial of patients with TD.

OTHER SCHIZOPHRENIA-RELATED STUDIES

The Twin Studies. It has been our pleasure for the past few years to host and participate in a study conducted by Drs. Torrey and Gottesman. The subjects of their study comprise a group of twins with a majority who are discordant for schizophrenia. With a method similar to one he has been using to study the chronic schizophrenic patients who enter the Neuropsychiatric Research Hospital, Dr. Taylor has been collecting information on the developmental histories of these twins. Several interesting subgroups have been found. For example, by age five, one twin had clear cognitive and behavioral problems, while the other had no dysfunction. A small population of twins that developed

schizophrenia continued soiling and wetting their clothing after five years of age. This characteristic was not found in any of the normal controls or discordant well twins. A review of school performance prior to onset indicated that the population with schizophrenia was seen as less motivated, more often physically tired, and more behaviorally disruptive than the well twins.

One finding from the study has received a great deal of attention. In collaboration with the Clinical Brain Disorders Branch, Dr. Suddath found a decrease in the size of the anterior hippocampus, and an increase in the size of the lateral and third ventricles in the twin with schizophrenia compared with the normal twin. In 12 pairs, the affected twin could be identified through visual inspection by comparing the size of the cerebral ventricles. Correlations are being examined between these quantitative measurements and data gathered from clinical histories and from neuropsychological tests. This study strongly suggests that an environmental event is required for the development of schizophrenia.

Evoked Response Studies. Drs. Egan, Kirch and Suddath have been collaborating with Drs. Duncan and Mirsky in the Laboratory of Psychology and Psychopathology in a study that examines the correlation between temporal lobe structural abnormalities and the P300 abnormality in patients with schizophrenia. A previous report using a small number of patients found a relationship between temporal deficits in the P300 and several structural abnormalities on CT scans. This study has found correlations between temporal lobe structural abnormalities and P300 deficits. This suggests that information processing deficits,as reflected by the P300, are related to temporal lobe pathology in patients with schizophrenia.

Cost of Schizophrenia. Dr. Taylor, through a series of interviews and analyses, has made a detailed estimate of the cost of schizophrenia in the United States for 1991. Schizophrenia annually costs over $60 billion. Direct treatment and care cost about $28.4 billion, and lost productivity accounts for an additional $21.3 billion dollars per year. This information is being collected to help policy makers, professional mental health providers, and civic leaders understand better how schizophrenia economically affects the nation, and it will highlight the important role research can play in reducing both human suffering and the cost of treating individuals with this and similar illnesses.

For example, in contrast to the cost of treating patients with schizophrenia, the cost of treating patients with bipolar illness has dramatically decreased since the introduction of lithium. We have calculated that since 1969, lithium treatment for bipolar illness has resulted in a savings of over $39 billion in the United States alone. Reduced requirements for hospitalizations account for $11 billion (28%) of the total savings. Without the introduction of lithium, Social Security payments (SSI and SSDI) to patients would have been increased by at least $1.6 billion over the same 20-year period. Lithium has not only directly decreased medical costs, but has also allowed individuals to enter the work force and

become self-sufficient. This increased productivity by individuals with bipolar illness has added approximately $27 billion to the American economy.

Unfortunately, the current treatments for schizophrenia fail to significantly increase patient productivity. After the onset of schizophrenia, most individuals remain either unemployed or underemployed. Similar circumstances were previously true for patients with bipolar affective disorders. Nevertheless, once medical science introduced lithium, a low-cost treatment, fewer hospital inpatient days were required and productivity dramatically increased. Our report provides a comprehensive baseline for annually projecting direct and indirect schizophrenia treatment costs, and concretely measuring savings produced by the introduction of new treatments or prevention.

BRAIN GRAFTING AND PLASTICITY OF STRIATAL AFFERENTS

Parkinson's disease is caused by the degeneration of a small group of dopamine-containing neurons which are located in the substantia nigra (SN) and project to the corpus striatum. These ascending neuronal circuits are of crucial importance in the regulation of motor function, feeding and drinking, and other behaviors that are deficient in Parkinson's disease. It has been hypothesized that if tissue from the adrenal medulla, a region with an abundant supply of dopamine-producing neurons, is transplanted into the substantia nigra of Parkinson's patients normal functioning can be restored.

In testing this hypothesis, researchers have found that bilateral SN lesions in the rat brain produce pronounced behavioral abnormalities, including severe deficits in eating and drinking. Adrenal medulla and SN grafts into the brain seem to alleviate some of the manifestations of the SN lesions. Dr. Freed and his section are now conducting several experiments to investigate the factors that control the efficacy of these grafts, and, in the case of adrenal medulla grafts, to investigate how grafts influence brain function.

One series of experiments used the intraventricular SN graft model to study the factors which limit the efficacy of these grafts. As several studies in the rat have reported that immature corpus striatum is the preferred target for developing dopaminergic neurites in tissue culture, it was hypothesized that the major factor limiting reinnervation of the host brain was the adequacy of the mature host striatum as a target tissue. When embryonic striatum was transplanted into the host brain in combination with SN, the transplanted striatum was entirely reinnervated to the exclusion of the denervated host striatum. This suggested that the immature striatum is a "preferred" target for reinnervation, while reinnervation of the mature host brain is limited. To translate this finding to a functional model, SN (or sciatic nerve control tissue) was transplanted into normal immature hosts on the first day after birth. The hosts were allowed to mature, and then received bilateral SN lesions. Animals that had received SN grafts were substantially protected from the effects of the SN lesions; these

animals showed increased eating, drinking, and activity, and appeared less rigid than sciatic nerve controls. The changes in eating and drinking were particularly interesting because it has generally not been possible to influence eating and drinking with SN grafts in mature SN-lesioned animals. These data suggest that the functional effects of SN grafts may be primarily limited by the adequacy of mature striatum as a target tissue. In contrast, varying the age of the embryonic donors had a limited effect on SN grafts. The optimal age was 15 days, but further decreases in donor age resulted in no greater functional effect. In fact, grafts from 11 and 13 day gestational donors required long delays (12 weeks) for the appearance of maximal functional changes. Thus, age of the host, rather than age of the donor, appears to be the major factor influencing the success of intraventricular SN grafts.

Dr. Freed found previously that adrenal medulla grafts transplanted to the lateral ventricle also decrease apomorphine-induced rotational behavior. Such grafts are effective, however, only when taken from relatively young donors. Along with others, we have also observed that adrenal medulla grafts do not survive well when transplanted into the parenchyma of the striatum, and correspondingly, the behavioral effects of intrastriatal adrenal medulla grafts are small. Intraventricular adrenal medulla grafts in neonatal animals were found to produce some increase in eating and drinking following bilateral SN lesions, but these effects were much less than those produced by SN grafts. With Dr. Poltorak and Dr. Becker (University of Michigan), we continued to investigate the mechanisms of action of adrenal medulla grafts. These grafts did not increase CSF dopamine concentrations, but did cause increases in dopamine in serum. The increases in serum dopamine correlated with the behavioral effect of the grafts, but, curiously, not with survival of the transplanted cells. Using dialysis probes, Dr. Becker has also observed contralateral changes in brain dopamine release after unilateral adrenal medulla grafts. Dr. Takashima was recruited to pursue experiments to clarify the meaning of these findings. He has found that adrenal medulla grafts induce two separate and independent effects. One effect, which is responsible for a part of the functional change, is specific for adrenal medulla grafts and may be related to secretion of catecholamines by the transplanted cells. The second effect is a nonspecific response to surgery which appears to be related to changes in blood dopamine via the host adrenal gland.

Drs. Poltorak and Freed have studied the expression of a series of cell adhesion molecules (CAMS) (L1/Ng-CAM, N-CAM, J1/Tenascin molecules, and MAG and their common L2/HNK-1 epitope) in normal rat adrenal gland sections as well as in adrenal medulla cell cultures, with and without NGF stimulation. In situ, L1/Ng-CAM and N-CAM immunoreactivity was present on chromaffin cells and in surrounding connective tissues. The extracellular matrix of whole medullae also expressed J1 molecules. In long term cultures, NGF stimulation enhanced both L1/Ng-CAM and Thy 1.1 immunolabeling on chromaffin cells and their processes. NGF-activated chromaffin cells also demonstrated neurofilament and vimentin-like immunoreactive filaments within cell bodies

and their processes. Chromaffin cells were usually found on a layer of N-CAM as well as ECM laminin process outgrowth from chromaffin cells.

They have also studied the expression of CAMs in adrenal medulla fragments implanted into the lateral ventricle. Surviving implanted chromaffin cells showed enhancement of surface L1/Ng-CAM expression as compared to normal chromaffin cells in adrenal medullae. The implanted chromaffin cells demonstrated only a partial conversion to neuronal phenotypes. Surviving chromaffin cells were accompanied by a reorganization of their closely associated extracellular matrix (ECM). As compared to normal, in situ adrenal medullae, graft ECM demonstrated a substantial increase in L1/Ng-CAM and laminin immunoreactivity and a decrease in J1/tenascin expression. Some adrenal medulla grafts degenerated, and, in these cases, grafts showed a fragmentation of ECM and a gradual disappearance of CAMs. These results suggest that surviving adrenal medulla grafts exhibit an increased synthesis of certain CAMs by chromaffin cells which may be involved in interactions between chromaffin cells and the surrounding ECM. It is speculated that both surviving and degenerating adrenal medulla grafts could provide CAM and ECM components, including laminin, to host brains and, in this way, contribute to the functional effects of grafts.

Drs. Freed and Poltorak have also been employing in vitro methods to examine the effects of various substrates of in vitro extension of neurites from embryonic, dopaminergic neurons. They have found that L1/NgCAM is the most potent substrate, followed by fibronectin and laminin. Other molecules, including L2, meylin-associated glycoprotein, poly-L-lysine, and RGD attachment peptide, were less effective or ineffective. These studies will be compared with in vivo experiments to define the role of extracellular matric and cell surface protein expression in the differentiation and growth of dopaminergic neurites.

These studies are intended to examine the relationship between properties of the brain milieu as a substrate for neurite extension, and development of dopamine-containing fibers derived from SN grafts. In an initial experiment, cortical lesions were found to increase the growth of fibers from grafts into the host brain, but only in the most dorsal part of the striatum, close to the lesioned brain area. Reinnervation of other parts of the striatum was not changed by lesions. Results of a long-term study also support this conclusion. These differences were not due to an anatomical distortion of the brain from the lesions or to other anatomical artifacts. The cortical lesions themselves were also found to reduce rotational behavior by substantia nigra grafts. In another experiment, we found that kainic acid lesions of the striatum also stimulated reinnervation of the striatum by substantia nigra grafts. In contrast to cortical lesions, kainic acid lesions of the striatum did not interfere with the behavioral effect of grafts. Studies of cell survival in substantia nigra grafts and effects of other types of lesions are continuing. In these studies, we are now exploring the relationships

between changes in the expression of extracellular and cell surface proteins and the extension of dopaminergic neurites from substantia nigra grafts.

Drs. Poltorak and Freed have been conducting a series of experiments to examine effects of striatal deafferentation on the expression of molecules of the surface of striatal cells. The long-term goal of this research is the development of a model for the control of cell surface protein expression and consequent circuit remodeling, as influenced by the removal and/or activation of striatal afferents. As described above (number 1), cortical lesions increase the ourgrowth of catecholaminergic neurites from substantia nigra grafts into the deafferented striatum. Initial experiments showed that lesions of the frontal motor cortex increase expression of the cell adhesion molecules L1 and N-CAM, and decrease tenascin expression in the deafferented parts of the striatum. A subsequent study to examine the time-course of these changes after lesioning and regional specificity of the changes, and to develop an improved calibration method for the measurements, is in progress.

Embryonic brain tissue allografts, under many circumstances, survive transplantation into the brain. It is generally believed that such grafts will not survive if the host animal is systemically sensitized, by skin grafting or other means, to MHC antigens of the donor animal. Drs. Polotorak and Freed have found that Fisher 344 brain grafts survive in Brown-Norway host rats even when the host is systemically sensitized to Fisher 344 tissue. Embryonic, cerebral neocortices from Fisher 344 donors were transplanted into Brown-Norway host rats. Subsequently, the host animals were systemically sensitized with donor skin, brain tissue, or spleen cells, and compared to a control group consisting of allografts with no sensitization or sham procedures. Rejection of the transplants in Brown-Norway rat hosts was not provoked by any of the sensitization methods tested. Minor immunological responses that did not result in rejection were, however, present in many host animals. This increase of MHC class I expression was not accompanied by an infiltration of cytotoxic T cells. Thus, the findings suggest that neural graft rejection depends on general genetic susceptibility to immune reactions, and not only on disparity between donor and host antigens encoded by the MHC. Moreover, enhancement of MHC class I and class II expression within transplanted tissue does not predict graft rejection.

This finding raised the possibility that, in brain tissue transplantation paradigms, systemic sensitization does not invariably result in allograft rejection. We have therefore transplanted fetal cerebral cortex from either Lewis or Brown Norway donors into the brains of several isogenic and allogenic rat strains in order to determine whether (i) there are specific host-donor strain combinations that allow continued brain allograft survival following systemic sensitization, and (ii) whether such resistance is attributable primarily to the donor or to the host strain. We found that, even following systemic sensitization, brain grafts from Lewis strain donors survived in most host strains, whereas grafts from BN donors were rejected in all allogenic strains. Therefore, elimination of

immunological privilege by systemic sensitization does not always occur. Despite complete genetic disparity, for some strain combinations, brain allografts can survive after systemic sensitization. For the majority of strain combinations when Lewis was the donor strain, systemic sensitization did not produce complete graft destruction, whereas all allografts from BN donors resulted in severe immune responses. These results show that systemic sensitization is not invariably followed by complete rejection of brain allografts. Immunogenicity of the donor seemed to be the single most important factor in predicting this resistance to rejection.

The research program of Drs. Freed and Poltorak has two long-term goals: (1) Efforts will be devoted to the examination of effects of defined and genetically altered cells following transplantation, to better understand the behavioral effects of brain grafts and to develop this technique as a means of altering the functioning of the injured brain. (2) Immunostaining and in situ hybridization methods will be employed in combination with embryonic brain tissue transplantation to define the factors which control plasticity and cell adhesion events in nigrostriatal and corticostriatal systems.

CONCLUSION

Dr. William Freed served as program chair, and chair of the International Organizing Committee for the Fourth International Symposium on Neural Transplantation, held in Washington D.C. in July. It was a great success. He and the other members of his section (especially Mrs. Cannon-Spoor) are to be congratulated for their excellent work. Dr. Freed was asked to serve as Editor-in-Chief for the Journal of Neural Transplantation and Plasticity, and serves on the editorial boards of several journals, including Regional Immunology, Restorative Neurology and Neuroscience, Cell Transplantation, and Experimental Neurology. He was also invited to write an editorial for the Journal of Neurosurgical Anesthesiology. His work on neural tissue transplantation and on functions of glutamatergic systems has been recognized by invitations to give lectures at the American Society for Artificial Organs, the Behavioral Sciences Meeting, and John Hopkins University.

Dr. Henrietta Kulaga's exploration of the laboratory rabbit as a system for development and screening of HIV-I and HTLV-I vaccines and therapeutics has led to invitations at a number of important scientific meetings. This past year, the rights to use her patent, "The rabbit as a model for HIV-I infection", were purchased by several corporations.

Dr. Janice Stevens continues to visit the family welfare projects she started in three areas of India; these projects are supported by Hewlett Buffet foundations and have received praise from a recent World Bank report. A report was published on this important project in Family Planning (May-June 1992). She has

also been appointed an honorary member of the scientific advisory board of Charles University in Prague.

Dr. Rif El-Mallakh was given a NARSAD Young Investigator Award for his work on tardive dyskinesia. Dr. Magda Giordano won the Mead Johnson Travel Award.

In the last year, I was appointed to the American Psychiatric Association Task Force on Practice Guidelines for Treating Schizophrenia, and the Scientific Advisory council of NARSAD. I remain on the Scientific Committee of the National Alliance for the Mentally Ill. I am also on the executive scientific board of the National Depressive and Manic Depressive Associations, and Chairman of the Board of the Manic Depressive Illness Foundation. During the year, I was awarded the Bronze Medal (out of 300 entries) by the British Medical Association for my role as coordinating producer of the PBS special, 'To Paint the Stars'. In addition, I received the Bell Media Award from the Mental Health Association of Atlanta, and was listed in the Best Doctors in America. I was also awarded the NIMH D/ART Media Award. I was appointed to the World Bank task force on the cost of mental illness and gave the Veterans Administration First Annual Clinical Neuroscience lecture.

During the last year we were sorry to lose several staff members: Geoffrey Jackson, our first Stanley Scholar, is now at Hahnemann Medical School; Bonnie Dodd is working in Denver; Rif El-Mallakh, M.D. became the Director of the Affective Disorders Clinic at the University of Louisville, Kentucky; Gad and Varda Gilad returned to Israel to work at Technion-Israel, Inst. of Technology. Darrell Kirch, M.D. has been promoted to Deputy Director, Division of Clinical Research.

We are pleased that Ivory Baker, Ioline de Saint Ghislain, Doug Feltner, M.D., and Ben Grasso, M.D. have joined us this year.

For a number of years, it has been my privilege to work with many talented people. I feel very fortunate that this was true again.

October 1, 1991 to September 30, 1992
Laboratory of Biochemical Genetics
Carl R. Merril, M.D., Chief

The Laboratory of Biochemical Genetics is primarily concerned with diseases associated with aging, particularly those diseases affecting the central nervous system (CNS). As many of these diseases which affect the central nervous system are of unknown etiology, the initial approach to understanding these diseases is generally awkward. Researchers have been able to achieve considerable success utilizing biochemical techniques to search for abnormal pathways. This direct approach is limited to both our level of understanding of biochemical pathways, and sufficient knowledge of the disease to provide a clue as to which pathways to investigate. However there are large numbers of genes of unknown function.

The Laboratory of Biochemical Genetics has focused a major portion of its effort on the development and use of non-biased tools. In general, a non-biased tool can be defined as a tool that does not require prior information about the specific pathways involved. High resolution 2-dimensional electrophoresis falls in this category as does genetic linkage mapping with highly polymorphic linkage probes. The Laboratory has spent a considerable effort to develop high resolution protein electrophoresis, both in the running of the gels and in their analysis. We are currently capable of identifying over 3,000 proteins from samples of tissue or cultures. Analysis of this number of proteins provides information on greater than 2 megabases of coding DNA. It also allows examination of post-translational events and the expression of exogenous genes such as viral genes. We are using this technology to search for protein variation in disease states and also to study physiologic events such as the complex protein variations which regulate circadian rhythms.

Several years ago, Dr. Harrington, in this Laboratory, identified a set of protein spots that were present in Creutzfeld-Jakob disease, schizophrenia and herpes encephalitis. These proteins are present in 1/3 of the patients with schizophrenia. This observation was confirmed last year by Wildenauer et. al., in Munchen, Germany. This group also sequenced these proteins, demonstrating that they are fibrin fragments of fibrinogen. In an independent study, we recently verified the presence of these spots in approximately 1/3 of the schizophrenia patients, as well as in CSF from a number of

Alzheimer's disease patients (AD). We also found an apparent increase in alpha 2 FS haptoglobin in CSF from both Alzheimer's disease and schizophrenic patients. Haptoglobin's main known function is to bind hemoglobin for the scavenging of iron. It is often increased in iron deficiency anemia, however the hospital records provided no indication that the patients in this study had iron deficiency anemia. It may be of interest that haptoglobin and fibrinogen are both acute phase proteins. Acute phase proteins are increased in both chronic and acute inflammations. The presence of these proteins in spinal fluid may indicate an inflammatory state. However, there are several aspects of our findings that are not consistent with a simple acute phase response: 1) only the alpha 2 FS haptoglobin is increased, the other haptoglobin isoforms do not appear to be increased. 2) only fragments of fibrinogen are present.

The preferential presence of alpha 2 haptoglobin may indicate a genetic component in these diseases. The Laboratory is currently surveying schizophrenic, Alzheimer's disease patients, and normal controls to see if there is a genetic association of alpha 2 haptoglobin with the diseased individuals.

High resolution two dimensional electrophoretic investigations of plasma proteins have been more difficult than those the laboratory performed on CSF proteins, primarily because of the higher concentration of proteins in the plasma. A number of the plasma proteins have been identified by the Anderson's and their colleagues on high resolution

2-D electrophoretograms. This Laboratory continues to update these high resolution 2-dimensional protein maps. In addition, many of the proteins that can be visualized both in plasma and CSF have been studied in normal physiologic states and disease states. We have collected the literature from the past decade concerning these proteins and their quantitative alterations and are currently constructing a database to help us interpret protein alterations in diseases affecting the CNS. The database may give us some insight into the underlining pathophysiology of some of the CNS diseases of unknown etiology that we are currently investigating.

Drs. Wallace and Johnson of the Laboratory are characterizing altered proteins exhibited by Alzheimer's disease-afflicted brain tissues in an effort to understand their role in the pathology of the disease. Using the approach of visualizing altered proteins in AD tissues by two dimensional gel analysis, we identified two specific differences. First, we determined that the protein synthesis factor, elongation factor 2 (EF-2) was more highly phosphorylated in total homogenates from AD tissues. The phosphorylated EF-2 correlates with our previous observation that protein synthesis is drastically inhibited in AD brains. Second, examination of translation products from AD polysomes showed a dramatic elevation of heat shock 70 proteins (hsp 70). We are currently investigating the physiological consequences of the increased levels of hsp 70 in heat shocked neuronal PC12 cells. We have characterized a number of biochemical responses that have direct relevance to AD. First, a number of nascent polypeptides contranslationally form stable complexes with the induced hsp 70. This association is consistent with the role of hsp 70 as a cytoplasmic molecular chaperone. We have identified one as the cytoskeletal-associated protein tau. Further, with heat shock, tau is transformed into A68, the primary protein component of neurofibrillary tangles, by an abnormal phosphorylation. The abnormal phosphorylation appears to be mediated by protein kinase C and is prevented when tau associates with hsp 70. Interestingly, even with the transformation of tau to A68, the PC12 cells do not exhibit any evidence of tangle formation, at least in short term treatment. We have also shown that Amyloid Precursor Protein (APP) is phosphorylated by protein kinase C in these cells. This phosphorylation is dramatically reduced in the heat shocked cells.

We have used an animal model of AD to characterize the physiological function and processing of APP in the intact brain. We have found that various subcortical lesions of the rat brain result in the induction of APP in the cortex. The induction is specific to the APP gene, in that numerous other genes are not induced, and specific to the loss of subcortical innervation in that other disruptions of cortical function do not induce APP. The induction is rapid (1 hour post lesion) and persistent (45 days post lesion). This response suggests that a normal function of APP may be related to injury-induced synaptic plasticity. A more subtle and reversible lesion, by subcortical placement of lidocaine, results in a reversible induction of APP. Interestingly, although APP mRNA levels and biosynthesis are increased 3 to 4-fold, mature fully processed APP is not elevated. This increased turnover of APP indicates that the rat cortex can effectively catabolize the excess APP and most likely explains the absence of senile plaques in the lesioned rat brain. The lesioned cortex in wild type and transgenic animals will be used to determine if altered forms of APP may lead to production of senile plaques.

Dr. Joy, in collaboration with Dr. Mike Menaker's group, at the University of Virginia, has been studying protein alterations in hamster circadian rhythm mutants. Dr. Joy has been able to confirm protein alterations which appear to correlate with mutations that affect the circadiun rhythm in the hamsters. This observation extends the earlier work of Dr. Ann-Catherine Hochstrasser in this Laboratory. Efforts are currently underway to produce sufficient quantities of these proteins both in normal and altered

forms for protein sequencing. In addition, Dr. Joy is conducting studies to identify proteins involved in the maturation of circadian pacemakers and the initiation of circadian oscillations.

One of the curious aspects of diseases such as Alzheimer's disease is their association with aging. In addition, diseases such as schizophrenia appear more commonly during or shortly after puberty, a time when brain metabolism, and oxygen consumption is dramatically altered. There are some questions as to the relationship of these metabolic and/or aging alterations and the onset of the disease state. A number of investigators have begun to find accumulations of mitochondrial deletions in tissues which are associated with aging, and certain diseases, such as Parkinson's disease. Dr. Rath and I have investigated brain mitochondrial DNA for the presence of deletion mutants in young and old individuals. Our preliminary data seems to indicate that there is an accumulation of a deletion mutation encompassing approximately five thousand base pairs (BP), after the age of 27 years. This accumulation has been observed in individuals who were free of neurological disease. It may represent a "natural" aspect of aging which may precipitate or trigger events caused by other genetic or environmentally caused abnormalities.

The tools that the Laboratory is developing to map the human genome may provide additional support for our studies of somatic mutations. In this regard, the Laboratory continues to develop and characterized the highly informative microsatellite repeat polymorphic markers, and the sequenced tag sites from expressed brain genes. Our Laboratory has developed 55 microsatellite repeat polymorphisms and has placed them on human chromosomes using physical and genetic mapping methods. The Laboratory has emphasized the development of trinucleotide repeat polymorphisms and has developed rules to predict their informativeness based on the number of repeats. In addition, strategies for the rapid isolation of microsatellite markers from cosmid, phage, cDNA libraries have been developed.

Microsatellite markers have been utilized by this Laboratory in a number of gene linkage studies. The Laboratory participated in the mapping of the Treacher-Collins gene and Ushers type 2 gene. We have typed 90 markers in 171 individuals belonging to 34 families that segregate for schizophrenia. In addition, in collaboration with intramural and extramural scientists we have typed 80 markers in the old order Amish pedigree and have initiated a mapping collaboration for a bipolar gene in a large Canadian pedigree. Some of the polymorphic markers developed by the laboratory have been adopted by the FBI for use in forensics investigations.

The Laboratory has also determined the chromosomal location for 280 expressed brain genes. Mapping of these cDNAs has been facilitated by the use of somatic human-rodent hybrid-cell lines, and the polymerase chain reaction. These tools have provided us with an overall mapping success rate of over 80%. The problem of shared homologies between the human and rodent genome has been minimized using sequence tag sites (STS) from the three prime non-expressed regions of the cDNA's. These regions are less conserved than the expressed regions, and show greater genetic variability. We noted that, despite this increased genetic variability, approximately 10-20% of the STS's amplify sequences from the rodent template. Of these sequences we were able to map 2/3 of them as they varied in size from the sequence obtain from the human cDNA. The Laboratory hopes that by continuing to develop these molecular genetic tools, and utilizing them in diseases of unknown etiology that we will gain some insights that may help in diagnosis and in developing a rational therapy for these diseases.

The Neuropeptide group led by Dr. Yang has continued its efforts to characterize the involvement of neuropeptide FF(NPFF), a mammalian homologue of the molluscan peptide FMRF-amide. Opiate dependence studies in rat using superfused pituitaries and spinal cords, and icv injections strongly indicate that release of NPFF may participate in naloxone precipitated opiate withdrawal. In addition, a binding assay for NPFF has been developed in membrane preparations of rat brain and spinal cord. The neuropeptide group has also discovered and are in the process of isolating a neurite-outgrowth factor that is distinct from NGF, aFGF, bFGF, EGF, and IGF.

At the annual meeting of the Electrophoresis Society, one of the Laboratory's high resolution protein electrophoretograms, presented in a poster session by Dr. Joy, was selected as the outstanding two-dimensional electrophoretic gel of the meeting. Dr. Joy was awarded a certificate of honor by the Society and an illustration of the gel will be published in the next issue of the Society's Journal, Applied and Theoretical Electrophoresis.

In "non-project" related activities, the Laboratory Chief, Dr. Merril continues to serve the scientific community as the Electrophoresis Society's Editor-in-Chief, of the Journal Applied and Theoretical Electrophoresis. He also continues to serve as the ADAMHA representative to the Office of the Surgeon General, and as the Chairman of the Surgeon General's Advisory Panel for the PHS Commissioned Corps Research Officer Group. In these roles he provides representation and advocacy for the Agency and the research officers in the Commissoned Corps respectively. Dr. Merril was a major contributor to the establishment of the Research Officer Group (ROG) in 1989 to "provide an appropriate career track for doctoral-level individuals who are participating as researchers in programs of original research." He was recently awarded both the PHS Distinguished Service Medal and the Surgeon General's Exemplary Service Medal.

Annual Report - Clinical Brain Disorders Branch, DIRP, NIMH
October 1, 1991-September 30, 1992
Daniel R. Weinberger, M.D, Chief

FY 1992 was an eventful year for research activities in CBDB. A number of new areas of investigation were begun and promising developments from past activities continued. There were relatively few personnel changes. Michael Knable, M.D. joined us as a new senior staff fellow, having just completed his residency in neurology to add to his prior training in psychiatry. Dr. Annand Mattay, another recent graduate of the University of Virginia neurology residence, but also a board eligible nuclear medicine physician, is working in our lab as a fellow through the new radiology research fellowship program at NIH. Mary Herman, M.D. joined us as planned to add a unique level of expertise to the neuropathology section. To our chagrin, Laura Marsh M.D. left to become a fellow at Stanford so that she could marry her fiancee who is on the faculty there. We also bid a fond adieu to Daniel Press, our Howard Hughes fellow. Dr. Berman had a baby, Jonathan. Other than that, our numbers have remained stable.

A number of researchers in CBDB received special recognition again this year. Dr. Kleinman and Dr. Coppola received meritorious service medals from the USPHS. Dr. Berman received the commendation medal. Dr. Weinberger received the distinguished service medal. Dr. Kleinman became president elect of the Society of Biological Psychiatry. Dr. Hyde received a special award from the Tourette's Association.

Section on Clinical Studies

Neuroimaging studies have continued to focus on twin populations. We now have high resolution MRI volume scans on MZ twins discordant for schizophrenia, twins with bipolar disorder, twins with Tourette's syndrome, and normal MZ and DZ twins. We are continuing to make a variety of morphometric measurements on each of these twin data sets. So far we are elaborating on the findings in the discordant schizophrenia sets. The bipolar sets are being evaluated as well and the results of this component of the study should be available by the middle of FY 1993. The MRI data on the Tourette's twins has been disappointing, but refined methods are being applied to new sets with the hope that improved methods will provide more

informative results.

One of the interesting new application of MRI volume data sets has been to three dimensional surface and volume rendering. This is a computerized imaging method that produces detailed high fidelity renderings of the cortical surface. It is especially interesting in studying questions of hemispheric asymmetry and of developmental aspects of gyrification in identical twins. Preliminary results which are scheduled for oral presentation at this year's society of neuroscience annual meeting indicate that gyral development in normal MZ twins is under considerable experiential influence. The gyral patterns of normal MZ twins are remarkably dissimilar, a strikingly unexpected finding. We have begun to apply the method to questions of gyral development in schizophrenia, and other disorders. The method also has potential as an approach to registering volume data sets from other imaging modalities with MRI and for identifying surface structures that can then be projected back onto cross sectional images for more accurate quantification. Dr. Coppola has developed a new, automated threshold method for segmentation of cerebral tissue compartments that will be applied for quantification

Studies of brain function with neuroimaging methods has continued on several fronts. The newest method is using dynamic fast gradient echo MRI techniques. This work has been performed in collaboration with Dr. Joe Frank and the MRI research center at NIH. With gadolinium as a "tracer" of cerebral perfusion, it is possible to follow the "susceptibility" of intravascular and the immediate extra vascular space to minute changes in the local magnetic field caused by passage of the tracer through the tissue. Since the tracer is paramagnetic, it cause such local perturbations. The technical development that has made it all possible is the development of ultra fast scanning. A series of scans are taken less than every two seconds and tracer kinetic mathematics are applied to tracer concentration by time curves to determine functionally linked changes in cerebral blood volume. We have shown that with visual activation, a consistent increase in cerebral blood volume is found with the calcarine cortex. The method is soon to be applied to studies of cognitive activation. The potential advantages over nuclear medicine methods includes the lack of ionizing radiation exposure and the greater spatial and temporal resolution.

PET rCBF studies emphasize differential flow patterns during different cognitive and pharmacological states and in different neuropsychiatric conditions. Much of the energy of Dr. Berman and her team has been devoted to refining the methods of MRI - PET registration and of statistical

analysis. These are at time seemingly endless exercises, but their importance cannot be overemphasized. The PET study of MZ twins discordant for schizophrenia has been completed for the time being. The results confirm earlier data from two dimensional surface rCBF studies, in that the affected twin in every instance has less prefrontal rCBF than the unaffected twin during the prefrontally linked cognitive task, the WCS, but does not as consistently show this degree of discrimination during other tasks, even during difficult abstract reasoning tasks. We are continuing to explore the patterns of regional activation during more selective memory tasks to try to differentiate so-called frontal from mesial temporal memory systems.

rCBF during two different pharmacological treatment conditions in patients with schizophrenia has yielded some interesting results. Treatment with haloperidol and treatment with clozapine are compared during performance of the WCS and during performance of Ravens Progressive Matrices. The results in the first six patients indicate that in the basal ganglia the drugs are consistently different, regardless of the cognitive state. In the prefrontal cortex, however, the differences depend on the cognitive condition. The results illustrate how cortical function is critically state dependent. PET studies of the dopaminergic system have begun with F-18 fluorodopa. Fluorodopa is determined during the performance of the WCS in an effort to address the relationship of the tracer uptake to regional function and to actual performance on the test. Three medication free patients have been studies.

Our new SPECT scanner arrived in June with much fanfare and joy. After two years of struggling with the old scanner and with no success, we have moved completely to the new scanner. Preliminary experience with studies of rCBF and of Dopamine receptors have been very encouraging. Dr. Coppola has done an extraordinary job of getting the scanner operational and performing the check out, quality control procedures and of establishing the routines for research studies. While we were waiting for arrival of the new scanner, a study was completed in collaboration with George Washington University looking at specific and nonspecific binding with IBZM, a specific D-2 radioligand. We showed in comparing the active and inactive enantiomers that zeroing the striatal activity to the cerebellum was as good a method as zeroing it to the time activity data derived from administration of the inactive enantiomer. This finding has broad application in the clinical use of this neurochemical tracer. We have begun studies of D-2 receptors in normal twins and in twins with Tourette's

syndrome. Studies of patients with schizophrenia will begin shortly.

Dr. Sassaman and Lee have continued to perform excellently as experimental chemists. They developed rapid new methods for synthesis of I-Fisch, a promising new method D-1 radioligand, for IBZM, and for IQNB. In collaboration with Brian DeCosta's group at the NIH, a novel iodinated radioligand for sigma receptors has been developed and reported at the society of nuclear medicine annual meeting. Dr. Sassaman has been working on new imaging agents for the NMDA receptor and in collaboration with David Nutt of Bristol, England, a novel alpha-2 SPECT ligand.

The Tourette's syndrome twins studies under the outstanding direction of Dr. Thomas Hyde, who also runs the neurology clinic, continues to produce data about this disorder that are fundamentally changing our view of it. In studies of neuropsychology, psychiatric symptoms, and EEG it is clear that the pathophysiology of this disorder extends beyond that of the basal ganglia. Cortical and/or thalamic dysfunction is clearly implicated as well.

Neuropsychological studies of normal cognitive functions and of patients with Neuropsychological disorders is a top priority of CBDB. Dr. Goldberg and his group have continued to produce a series of important results from these efforts. In the past year, they have further explored the twin results, showing interesting relationships between neuropsychological abnormalities and MRI and rCBF data. The neuropsychology group has developed new paradigms for activation studies with PET, including a method for confounding attention during the WCS with a shadowing task. In a landmark longitudinal study of patients with schizophrenia from ages 20 to 80, they have shown that remarkable little progression of the neuropsychological deficits occurs, consistent with the notion that the neuropathological changes are fixed and not progressive. Dr. Gold has completed an unprecedented comparison of patients with schizophrenia and patients with temporal lobe epilepsy, to directly test the theory of temporal lobe dysfunction in the former. The results are interesting because they indicate that while the groups are fairly similar on memory function, the schizophrenics have poorer attention. This result is surprising because the patients with epilepsy had intractable seizure disorders and might have been thought to have had greater problems with attention.

Section on Neuropathology

There have been a number of important development in animal studies. Dr. Lipska has further expanded the neonatal rat excitotoxic hippocampal

lesion model of schizophrenia. This model is isomorphic with a number of aspects of schizophrenia and is much more promising than any animal model that has come before it. In a nutshell, the neonatally lesioned rats grow up looking indistinguishable from sham lesioned litter mates until after puberty when as young adults they become hyper responsive to novel environments, to stress, and to amphetamine. It appears that they grow into a condition of mesolimbic DA hyper responsivity. The emergence of the condition is blocked by neuroleptics. The potential of this model for understanding developmental mechanisms relevant for schizophrenia and for the invention of new drugs is very great.

To expand this model to the nonhuman primate, the application of the in vivo microdialysis method to study behaving rhesus monkeys has been a high priority. Dr. Richard Saunders and his team have made remarkable progress in this area. Extensive studies of DA release in the caudate and in the dorsolateral prefrontal cortex have been completed. These studies illustrate that DA concentrations are decreased by local administration of TTX and increased by local administration of potassium. Distant effects of local changes of DA concentrations have been confirmed for the first time in the monkey. Moreover, release dependent aspects of Glutamate concentrations have been demonstrated and preliminary data about local neurotransmitter concentrations in awake, behaving monkeys have been acquired. This is an extremely promising method for future studies.

In collaboration with researchers in the Neuropsychiatry Branch, Dr. Hyde and Dr. Kleinman have developed an interesting rat model of tardive dyskinesia. They have shown that after six months of haloperidol administration, persistent, unremitting dyskinesias develop in a large percentage of rats. In situ hybridization studies performed by Dr. Hurd have shown increased messenger RNA for various components of the opiate neurotransmitter systems of the basal ganglia.

There has been a dramatic reorganization of the neuropathology. With Dr. Herman joining the faculty of D.C. General Hospital, we have access to a large number of post mortem specimens. A pristine series of matched schizophrenia and control brains has been prepared for thin cryostat section and for receptor autoradiographic study as well as for in situ hybridization of various DNA and RNA probes. These studies are proceeding.

In collaboration with Jeffrey Joyce at University of Pennsylvania, Dr. Kleinman's group has replicated their prior finding of reduced presynaptic serotonin reuptake sites in prefrontal cortex of patients with schizophrenia. This is the first replication reported of this important finding. Other findings

from postmortem studies include increased 5-HT-2 receptors in basal ganglia of schizophrenics, and decreased choline acetyl transferase by western blot analysis in pontine tegmentum. Important negatives also emerged from postmortem tissue analyses, including a third negative study of cytomegalovirus DNA and of another negative study of cerebral gliosis which is important in further supporting the notion that the neuropathological changes are probably developmental in origin.